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Structure and Permeability Characterization of Sinojackia xylocarpa Hu drupe, based on High-field Magnetic Resonance Imaging, Scanning Electron Microscopy, Paraffin Section Detection

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Abstract

Sinojackia xylocarpa Hu is an endangered plant species endemic to China. In this study, we observed the permeability of Sinojackia xylocarpa Hu drupe in the imbibition phase by using magnetic resonance imaging (MRI) dynamics, and obtained the spatial representation of the water distribution in the drupe. At the same time, the structure of the drupe, the permeability of the seed coat and the endosperm were monitored through scanning electron microscopy (SEM), and paraffin section detection (PSD) .

Background

Sinojackia xylocarpa Hu is the first new genus published by a Chinese botanist, which belongs to the genus *Sinojackia Hu* of *styracaceae* family (Fig.1)[1]. It is a national key second-class endangered species[2]. At present, the wild trees are almost extinct, with only a few artificial cultivations in the botanical gardens of Nanjing, Shanghai, and Hangzhou. The *Sinojackia xylocarpa* Hu trees in Nanjing Zhongshan Botanical Garden were full of fruits, with a kernel rate of 84%[3]. The *Sinojackia xylocarpa* Hu trees in Hangzhou Botanical Garden were also lots of fruits, with a kernel rate of less than 20%[3]. The *Sinojackia xylocarpa* Hu trees in Nanjing Forestry University had full fruits for three consecutive years, with the kernel rate of 90%[4]. All the findings reflect that the *Sinojackia xylocarpa* Hu trees are fruitful and have many fallen fruits on the surrounding ground, but no sprouting seedlings are found. So the breeding of the *Sinojackia xylocarpa* Hu seeds is very difficult under natural conditions.

Results

2.1 Structural of the *Sinojackia xylocarpa Hu* drupe and seed

The drupe is oblong shape, with a dense pale brown lenticels surface, a pointed conical tip, a blunt circular fruit handle. From the structural point of view (Fig. 2), the drupe can be divided into exocarp, endocarp, seed shell, seed coat, and endosperm [9]. The exocarp was thin and keratinized, the endocarp was thick, soft and low in lignification. The seed was tightly packed in the endocarp. The seed is slender, the tip is long conical, and the tail is pointed conical. From the structural point of view (Figure 2II), the seed was divided into seed shell, seed coat, endosperm, cotyledon, hypocotyl, and radical. The seed shell was thick, hard and highly corky, the seed coat was thin and membranous, the embryo was enclosed in the endosperm and develops fully.

2.2 Spatial distribution of water protons

MRI was used to measure the spatial distribution of water in imbibition drupe and seed (Fig. 3, 3)[10]. MR images were obtained in axial orientations, coronal, which are parallel to the embryonic axis. The images presented in Fig. 3, 3 are limited to one 2D median slice in the coronal. The slices were taken as a series of sections from a single sample at a given time.

Fig.3I shows the MR image of the whole imbibition process of the drupe. After 4h of soaking, an intense water signal was observed in the fruit handle. Water moves initially through the fruit handle hole, then along the vascular bundle and endocarp enters the drupe. In images obtained after 13h,20h,28h,38h,48h,60h and 72h of imbibition, the water gradually penetrates into the exocarp[11]. An intense water signal was observed in the fruit tip after 82h of steeping. Then it was continued to imbibition for 97h,120h,138h,161h and 185h, moisture migrated from the fruit handle to the tip, while the water continued to permeate and move along the vascular bundle to the exocarp on both sides. During the next time of imbibition 21d, the unwetted area of the peel and the seed gets smaller and disappears completely after 31d. But the seed shell always has no red water signal. (Because of the use of AB glue to fix the drupe, the water can't enter the small part of the right side of the peel.)

Fig.3 exhibits the MR image of the whole imbibition process of the seed. After 4h,13h,20h,28h, 38h, and 48h of soaking, it can be seen that water entered from the seed tail and the seed tip, and the red water signal area of the seed cavity increased significantly. After 60h,72h,82h, and 97h of imbibition, the red water signal of the influent channel of the seed tail and the seed tip was obvious. After swelling for120h,138h, and 161h, the water gradually fills the seed cavity, and the gap between the seed coat and the endosperm. After 185h of inflating, the whole seed cavity, and the gap between the seed coat and the endosperm was completely filled with water. The seeds swelled obviously and filled the entire seed cavity. After 21d and 31d of imbibition, the seed tail, seed tip, and the whole seed cavity all showed an extremely strong red water signal. But the seed shell always has no water signal. However, the seed shell does not have any water signal during the whole imbibition process.

2.3 Tissue observation in scanning electron microscopy (SEM)

Observed by SEM, the tip of the drupe is convex (Fig. 4A). The fruit handle has an obvious hole (Fig. 4B) [12]. The surface of the exocarp is an irregular scaly stratum corneum (Fig. 4C)[13]. The endocarp is composed of large parenchyma cells, which are arranged loosely. The cell wall was full of small holes and the section likes honeycombs (Fig. 4D)[14]. The vascular bundles are linearly distributed longitudinally in the endocarp with large pores and gaps (Fig. 3E). The seed shell is composed of slender thick-walled cells with hollow interiors, arranged closely (Fig. 4F)[14]. The outer surface of the seed coat is arranged by irregular polygonal fibrous tissue, and there are a small number of small holes on the inner surface of the seed coat. (Fig. 4G)[16]. The outer surface of the endosperm has an irregular quadrilateral shape cells, which are closely arranged. The inner surface of the endosperm is uneven, and the cells are highly dense (Fig. 4H).

2.4 Tissue observation by paraffin sections

Paraffin sections were used to observe the morphological structure of the cells in the peel, shell, and endosperm[16]. The tip is closely arranged by parenchyma cells of different sizes(Fig.5A). The fruit handle is made up of closely packed thin-walled cells containing green cellulose cell walls(Fig.5B). The epidermis of the exocarp is distributed with lenticels and keratinized(Fig.5C). The mesocarp is composed of parenchyma cells of different sizes and shapes. The vascular bundle composed of numerous small

cells extends into the parenchyma cells of the endocarp(Fig.5D). The shell consists of closely arranged skeletal stone cells and round thick-walled cells of varying sizes, presenting a highly corked red color(Fig.5E).The endosperm is made up of closely packed and different sizes and shapes thin-walled cells containing green cellulose cell walls. The endosperm cells contain darkly colored round particles, and the number of particles in a single cell is different(Fig.5F). According to the determined experiment by further oil red O fat stain, iodine-potassium iodide protein stain, periodic acid-Schiff polysaccharide stain,we found that glycogen was distributed on the cell wall(Fig.5G), and a lot of fat (Fig.5H) and protein (Fig.5I)were distributed in the cell. The fat was hydrophobic, but the hydrophilicity of protein was very strong. In addition, dense endosperm cells create severe mechanical barriers.

2.5 Experimental studies on the germination characteristics of the embryos

The germination of the embryo was determined. After 24 hours, the whole embryo expanded significantly, and the cotyledons were slightly greenish. After 48 hours, the cotyledons turned green and the radicle showed signs of germination. At 96 h, the radicle was obviously elongated, the cotyledons were opened, and the whole embryo was germinated, and the germination rate was 100%. The results of germination experiments fully indicate that the embryos have no dormancy, this proved that the dormancy of the seeds may cause by the endosperm and seed shell.

Discussion

3.1 Permeability and mechanical characteristics of exocarp and endocarp

The anatomical picture showed that the seed is tightly wrapped in the exocarp and endocarp, and the exocarp and endocarp occupied a large volume. Therefore, the endocarp and exocarp may form a mechanical barrier to seeds. The PSD image showed that the exocarp is highly keratinized, and the MRI image showed that the water does not penetrate into the drupe from the exocarp. Thus, the permeability characteristics of the exocarp may very poor. In addition, the PSD images showed the large parenchyma cells of the endocarp were loosely arranged and the cell wall was full of small holes. The linear vascular bundles with large gaps inside were distributed longitudinally in the endocarp. The MRI images showed that water gradually penetrated into the endocarp through vascular bundles, which showed that the permeability of vascular bundles and endocarp was good.

3.2 Permeability and mechanical characteristics of the seed shell

During the dissection, we found that the seed shell was extremely hard and could only be cut through a chainsaw. Besides, the anatomical images showed the seed shell occupies a large volume, so the seed shell may form a mechanical barrier. The SEM images showed that the dense thick-walled cells in the seed shell were densely packed, and the PSD images showed that the thick-walled cells in the seed shell were highly lignified, while the lignified cells were impermeable and very hard[18]. Therefore, the seed shell has very poor permeability and forms a huge mechanical obstacle.

3.3 Permeability and mechanical characteristics of seed shell endosperm

Both anatomy and PSD pictures showed that endosperm occupied a large volume and tightly wrapped the embryo. The PSD and SEM pictures showed a dense arrangement of endosperm cells. The embryo germination pictures indicated that the embryos have no dormancy. Jia Shuguo found that the embryos can germinate smoothly after removing the endosperm from the radicle or cotyledon[5]. Thus, the endosperm formed a mechanical resistance to the embryo germination. The Fig5H showed that endosperm contained a lot of fat, and the fat was hydrophobic, but Fig5I showed that endosperm also contained a large number of proteins, and the hydrophilicity of protein was very strong. The Fig3F (viability test) showed that water can enter the embryo cavity through endosperm. Jia Shuguo found that 40% of embryos with intact endosperm had a 40% germination rate when cultured on the medium[1]. Therefore, the endosperm has certain permeability.

It can be seen that the mechanical obstacle of the endosperm may be an important reason for the embryo fails to germination.

Conclusions

In this paper, the fundamental reasons for the dormancy of the *Sinojackia xylocarpa Hu* seeds were revealed by studying the mechanical and permeability characteristics of peel, seed shell, endosperm. The mechanical and permeability barriers of seed shell and endosperm are the important reasons for seed dormancy.

The anatomy, SEM and PSD images showed that the hard and dense seed shell severely hindered the water penetration and formed a mechanical barrier. The dense endosperm cells which contain a lot of protein and fat have a serious mechanical obstacle to embryo germination. In addition, the smooth germination of the embryo indicated that there is no dormancy of the embryo.

Many studies have shown that the combined treatment of concentrated sulfuric acid + different concentrations of gibberellin + different germination conditions has a good effect on the germination of most dormant seeds. In 1999, Xiaohua Shi used acid etching 2D + Crack + Gibberellin 3 treatment + outdoor low-temperature stratification in winter method to obtain a germination rate of 40.06%, which is not ideal. Different from Shi Xiaohua's method, after the peeled seeds were etched for 1 day, we found that the seed shell was damaged and the endosperm was exposed (Fig.7). It is speculated that sulfuric acid may cause damage to the endosperm.

Therefore, in order to break dormancy, it is necessary to eliminate the permeability and mechanical barrier of seed shell and endosperm. The most critical step is to crack the seed shell, which is the supplement and enhancement of acid treatment. Besides, the gibberellin should be used to remove the mechanical barrier of the endosperm. What cracks method and how long acid etching will be the focus of our future research.

Methods

5.1 Plant Materials

Research permission on *Sinojackia xylocarpa* Hu tree has been obtained from Jiangsu Wildlife Protection Station. 1,000 experimental drupes were collected from the cultivated *Sinojackia xylocarpa* Hu trees on the campus of Nanjing Forestry University on January 22, 2019. Then the Southern Forest Seed Inspection Center of the State Forestry and Grassland Bureau tested the vigor and quality of the drupes. The drupes were stored at 4 ° C and 40% relative humidity. The 1000-kernel weight of fresh drupes measured by the weighing method was 576.3 g. The kernel rate of drupes is 92%. The initial moisture content of drupes and seeds was 22.46% and 7.67% respectively measured by the air oven method (ASBC 1992). The voucher specimen of this material has been deposited in the Southern Forest Seed Inspection Center of the State Forestry and Grassland Bureau, and the deposition number was 2019-cc-10.

5.2Measurement of scanning electron microscopy (SEM)

The scanning electron microscopy was used for the subtle analysis of the material morphology of *Sinojackia xylocarpa Hu*'s drupe[19]. The tissues taken from different parts of the drupe were fixed on the sample table with double-sided glue[20], then sprayed with a thin gold film on the surface, and observed under the scanning electron microscopy (FEI, American)[21].

5.3 Measurement of magnetic resonance imaging (MRI)

A high-field 7.0 T MRI apparatus (PharmaScan, Bruker Biospin GmbH, Germany) was used to observe the channel and distribution of water into the *Sinojackia xylocarpa Hu*'s drupe[22]. The drupe and the seed were fixed on the 25×75mm microscope slides in a 45ml centrifuge tube[23]. The tube was filled with water then inserted into the volume coil and measurements were carried out. The experiments were performed at a resonance frequency of 300.337 MHz at 22±1C. Proton intensity MR images were recorded with a RARE-PD. Anatomical images were acquired with a turbo-rapid acquisition relaxation enhancement (RARE) PD-weighted sequence (repetition time (TR)/echo time (TE) = 1033/10ms, slices =18, field of view (FOV) = 2.8 × 2.8 cm, number of averages = 15, matrix = 256 × 256, slice thickness/gap = 0.3/0 mm, flip angle = 180˚),scan time=12min, pixel resolution=109um[24]. Time-lapse images were acquired continuously at 23s intervals during the imbibition of the drupe.

5.4 Measurement of paraffin section detection (PSD)

The paraffin section detection was used to examine the cellular structural characteristics of *Sinojackia xylocarpa Hu* 's drupe. The tissues taken from different parts of the drupe were immediately fixed in 70% FAA fixative (Servicebio, Wuhan, China) for 24 h, then dehydrated through the ethanol series and finally embedded in the wax block. The wax blocks were sliced on a microtome with a glass knife to a thickness of 3 μm and stained with saffron and fast green[25], then observed under an optical microscope (Nikon, Tokyo, Japan)[26].

Declarations

Ethics approval and consent to participate

Legal and Licences statement

We strictly obeyed comply with the Convention on the Trade in Endangered Species of Wild Fauna and Flora, and also accordance with Law of the People's Republic of China on Wildlife Protection. Research permission on *Sinojackia xylocarpa* Hu tree has been obtained from Jiangsu Wildlife Protection Station.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

YBS conceived the original screening and research plans; YW performed the experiments using the MRI, SEM, TEM and PSD methods; YW designed the experiments and analyzed the data; YW conceived the project and wrote the article with contributions of all the authors. YW agrees to serve as the author responsible for contact and ensures communication. All authors have read and approved the manuscript.

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Figures



Figure 1

Digital photos of Sinojackia xylocarpa Hu tree, flowers, and drupes.

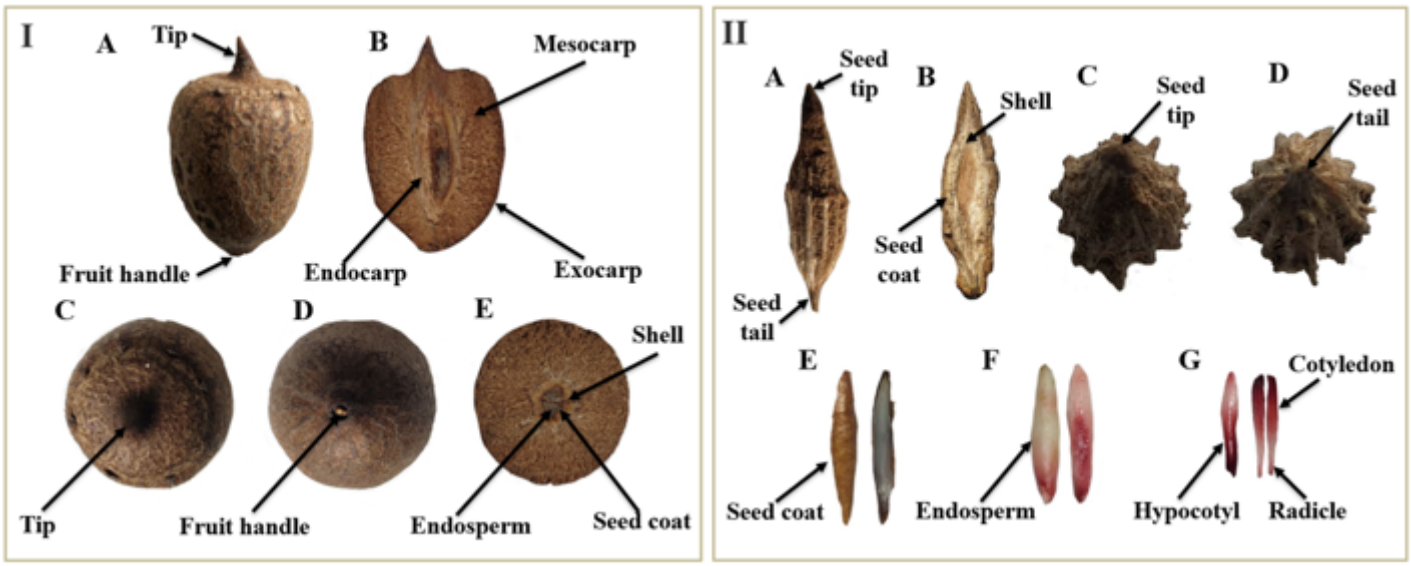


Figure 2

Digital photos of the drupe and seed structure.

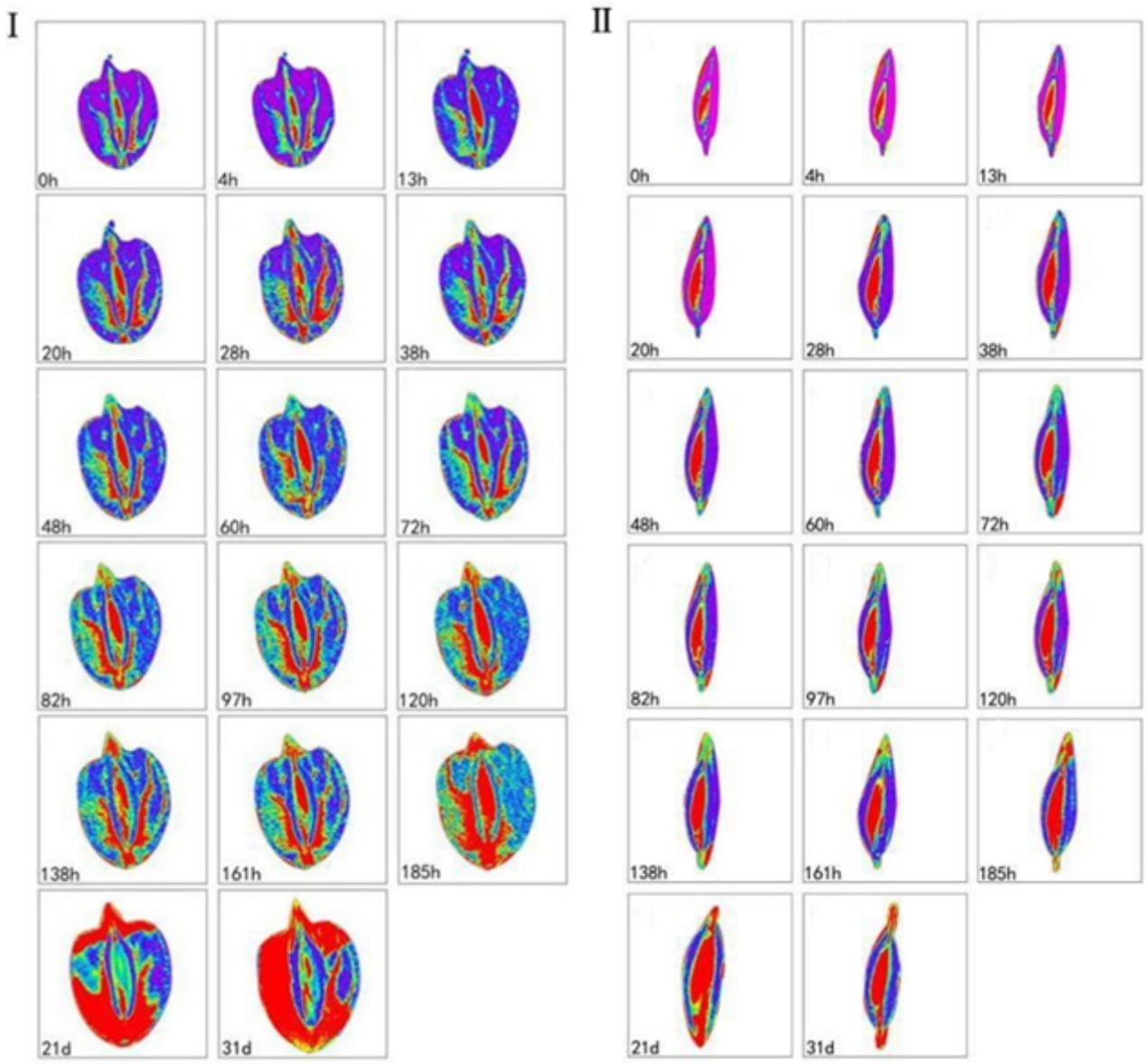


Figure 3

MR Images of the drupe, seed, and embryo at a median longitudinal section were acquired continuously for 744 h after imbibition, and the presented time is as follows: 0h, 4h,13h,20 h,28h,38 h,48h, 60 h, 72 h, 82h, 97 h,120 h,138 h 161 h, 185 h, 21d, and 31d.

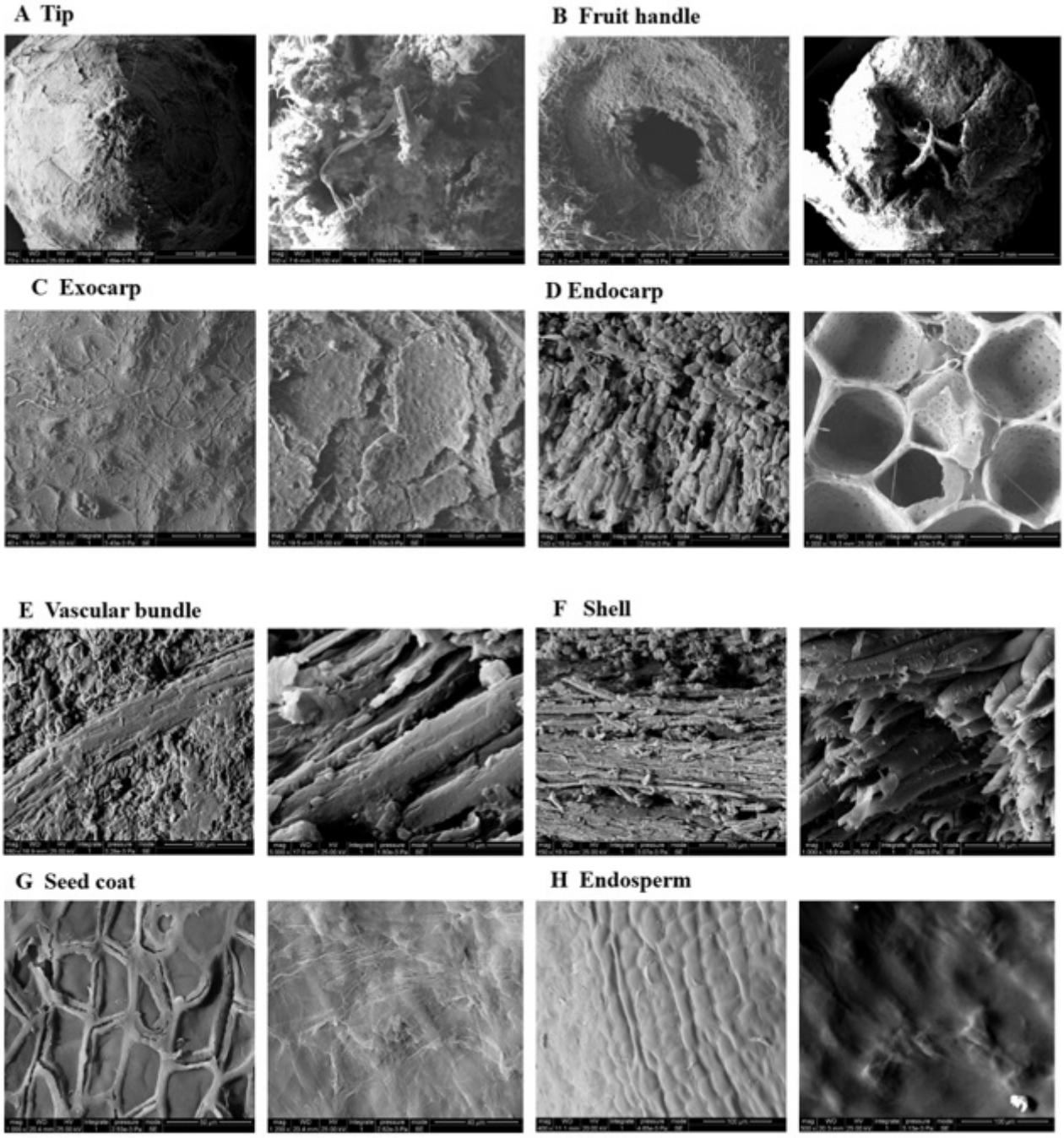


Figure 4

Scanning electron micrographs of the drupe, seed, and endosperm.

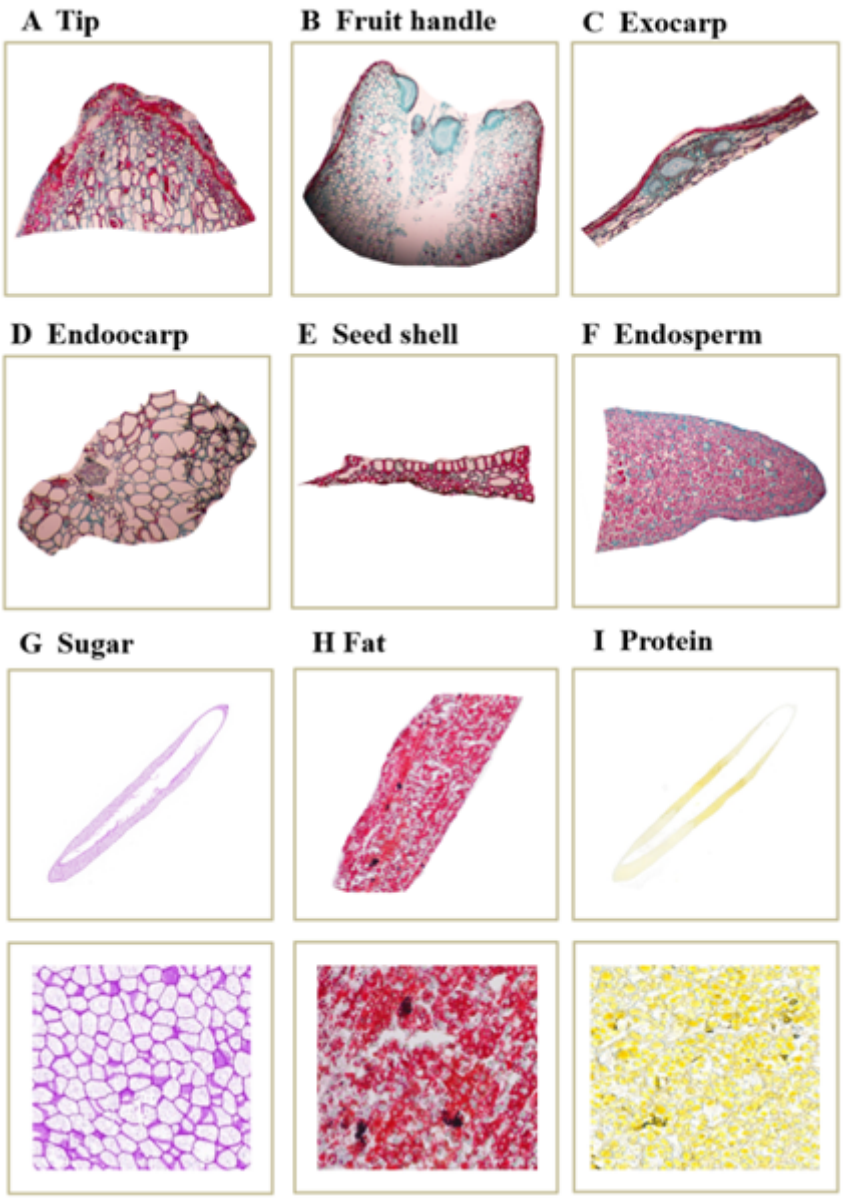


Figure 5

Light micrograph of parenchyma cells in a longitudinal-section of the drupe, seed, and embryo

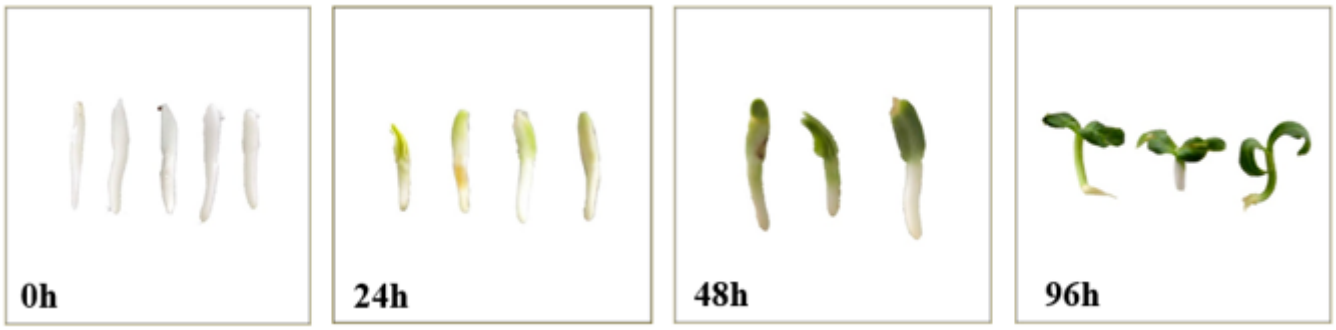


Figure 6

Digital photos of embryos germination at 0h, 48h, and 96h.



Figure 7

Digital photo of peeled seeds after acid etching.

Supplementary Files

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