

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

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Research article

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Abstract

• **Background** *Sinojackia xylocarpa* Hu is the first new genus published by a Chinese botanist, which belongs to the genus *Sinojackia* Hu of *Styracaceae*[1]. It is a national key second-class endangered species [2]. It is only distributed in the area near the area of Nanjing city, Jiangsu Province. The wild plants are almost extinct because of the deep dormant habit of seeds and artificial destruction. At present, there are only a few cultivations in botanical gardens in Nanjing, Shanghai and Hangzhou [3]. In addition, the *Sinojackia xylocarpa* Hu tree has white and lovely flowers, and shaped like hammer fruits, which have very special features and high ornamental value[4] (Fig. 1). Due to the lack of an in-depth understanding of the biological characteristics and dormancy causes of its seeds, the progress of artificial breeding is very slow. Therefore, in order to protect this endangered tree species, it is necessary to carry out research on the dormant mechanism and reproduction of seeds. In this paper, the MRI, SEM, TEM and PSD test methods were used to study the structure and permeability characteristics of the drupe, and explore the mechanical and permeability barriers of peel, seed shell and the contents of endosperm [5, 6]. According to the experimental results, we found important reasons for seed dormancy, and put forward suggestions for how to break the dormancy, in order to provide a theoretical basis for the seed large-scale reproduction of *Sinojackia xylocarpa* Hu tree.

• **Result** We observed the water channel and distribution through MRI in the imbibition phase, and found that the fruit handle hole and the fruit tip are the main entrance for water to enter the drupe. After entering the drupe, water penetrated into the peel along with the vascular bundle. This illustrated that the exocarp, the mesocarp, and the endocarp are all with good permeability characteristics. The SEM, TEM, and PSD showed that the shell was highly lignified and corky, and there were a large number of high electronic dense substances in the endosperm. This demonstrated that the shell and the endosperm have poor permeability and caused mechanical barriers to seed germination.

• **Conclusion** The peel has good water permeability but has mechanical obstacles. The hard seed shell caused mechanical obstacles, and the permeability is poor. Neither the peeled seeds nor the seeds with partial endosperm germinated, but the embryos germinated normally. It can be speculated that dense seed coats and endosperms with high-fat content may cause mechanical obstacles. And the endosperm has poor water permeability. It can be seen that mechanical barriers and water permeability problems are the main reasons for the dormancy of the *Sinojackia xylocarpa* Hu drupes. Therefore, it is particularly important to remove mechanical barriers and permeability barriers of the drupes. Next, we will explore how to reduce the mechanical barriers and permeability barriers of the pericarp, seed shell, seed coat, and endosperm by sulfuric acid treatment, and accelerate the process of dormancy release by low-temperature stratification.

These main limiting factors of drupe germination will provide a theoretical basis for understanding the root cause of its dormancy, and put forward targeted opinions on the research direction of breaking the dormancy of *Sinojackia xylocarpa* Hu drupes, and provide a scientific basis for further exploring how to

release dormancy and expand seeding and reproduction. It also will have great significance for the phylogenetic of the entire benzoin family.

2. Results

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

The drupe of *Sinojackia xylocarpa* Hu is oblong shape, with a dense pale brown lenticels surface, a pointed conical tip, a blunt circular fruit handle, and a thick cork. From the structural point of view (Fig. 2A), the drupe can be divided into exocarp, mesocarp, endocarp, outer seed coat, inner seed coat, endosperm[7]. The seed of *Sinojackia xylocarpa* Hu has a hard shell, thick membranous coat and brown color, contains 1-3 pieces of fine-column seed embryos. The seed tip is long conical, and the seed tail is pointed conical. A thin layer of the inner seed coat, light brown, tightly wrapped in the endosperm. From the structural point of view (Fig. 2B), the seed can be divided into outer seed coat, inner seed coat, endosperm, cotyledon, hypocotyl, radical.

2.2 Tissue observation in scanning electron microscopy (SEM)

Observed by SEM, the tip of the drupe is raised (Fig. 3A). The fruit handle has an obvious hole, and the hole is associated with water entry into drupe (Fig. 3B)[8]. The surface of the exocarp is an irregular scaly stratum corneum composed of densely packed thick-walled cells. There are a small number of minute cracks in the recessed portion (Fig. 3C)[9]. The mesocarp is grayish-white, and the surface is mostly linear, semi-circular and torn, composed of large parenchyma cells (Fig. 3D)[10]. The endocarp consists of honeycomb-shaped parenchyma cells. The cells are large and relatively obvious. They are loosely arranged, with pores connected in the middle, with lateral vascular bundle distribution, low degree of lignification, and a highly elongated palisade tissue layer. The cells are arranged loosely and have distinct cell gaps (Fig. 3E).

The shell is elongated, gray, boney, like a jujube nucleus, with a highly corked surface, and uneven, irregular longitudinal edges, which are difficult to separate from the endocarp. The surface cells are papillary bulges, arranged neatly and tightly. The outer cells are fibrous thickened. The inner cells develop into thick-walled tissues, and the shell is obviously thickened from the outer to inner cell walls (Fig. 3F) [11]. The seed coat is thick and membranous, with a tan color, densely arranged cells, uneven surface, and tightly close to the shell. The membranous seed coat has a strip-like fibrous tissue, which interlaced and arranged in a woven pattern and has a fine structure, with fine cracks and small holes on the surface of the seed coat (Fig. 3G)[12]. The outer surface of the endosperm has an irregular quadrilateral shape, which is closely arranged, the inner surface of the endosperm is uneven, and the cells are highly dense (Fig. 3H).

2.3 Spatial distribution of water protons

MRI was used to measure the spatial distribution of water in imbibition *Sinojackia xylocarpa* Hu drupe, seed and embryo (Fig. 4A, 4B, 4C)[13]. MR images were obtained in axial orientations, coronal, which are parallel to the embryonic axis. The images presented in Fig. 4A, 4B, 4C 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.

2.3.1 Spatial distribution of water protons in *Sinojackia xylocarpa* Hu drupe

Fig.4A shows MR images of dry *Sinojackia xylocarpa* Hu drupe. The initial moisture content of the mature dry drupe was 22.46% and can readily see in the coronal plane. The water distribution is inhomogeneous, and mainly distributed in the vascular bundle and embryonic axis. 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 mesocarp and exocarp[14]. 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 mesocarp and 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 coat 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.4A(0h) shows MR images of dry *Sinojackia xylocarpa* Hu embryo were taken from the MR images of the drupe and amplified. From the longitudinal section of 0h, the initial moisture of the embryo is mainly distributed along the center of the embryonic axis. When inflated for 7B(4h), the radicle and hypocotyl showed a red water signal. When the swelling was 7C(13h), the cotyledon showed a red water signal. After 7D(20h), 7E(28h), 7F(38h), 7G(48h) swelling, the whole embryo showed obvious red water signals. After 7H(60h), 7I(72h), the water enters from the radicle and the small hole in the lower right end of the endosperm. After swelling for 7J(82h), 7K(97h), 7L(120h), 7M(138h), 7N(161h), 7O(185h), the whole embryo has a significant red water signal, and the volume of the embryo is gradually increased largely. After inflating 7P(21d) and 7Q(31d), the embryo was enlarged to the entire embryo cavity, and the channel of moisture entering the lower right side of the endosperm is visible.

Fig.4A(0h) shows MR images of dry *Sinojackia xylocarpa* Hu seed. From the coronal plane, the initial moisture of the dry seed is mainly distributed in the embryo, sporadically distributed in the seed tip and tail, which was 7.67%. After 9B(4h),9C(13h), 9D(20h), 9E (28h), 9F(38h)and 9G(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 embryo of the seed cavity increased significantly. Water entered from seed tip penetrated into cotyledon, while water entered from seed tail, then moved to the hypocotyl from both the right lower pore of the endosperm and the radicle. After 9H(60h), 9I(72h), 9J(82h) and 9K (97h) of imbibition, the red water signal of the influent channel of the seed tail and the seed tip were obvious. After swelling for 9L(120h), 9M(138h), and 9N(161h), the water gradually fills the seed cavity, and the gap between the seed coat and the endosperm.

After 9O(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 9P (21 d) and 9Q (31 d) of imbibition, the seed tail, seed tip, water intake channel, 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.

Figure 5A shows the change of SNR in different areas of the drupe during 31d imbibition time. Changes in the intensity of the MRI SNR data generally show three distinct stages of the imbibition: an imbibition phase I, an imbibition phase II and a saturation phase. Five areas in the MR images were integrated separately in Figure 5A: the fruit handle region (black solid square data set), the exocarp region (red solid circle data set), the mesocarp region (blue solid triangle data set), the endocarp region (green solid inverted triangle data set), and the tip region (purple solid rhombus data set). The SNR intensity of the five parts of the drupe was compared as follows: exocarp > fruit handle hole > endocarp > fruit tip > mesocarp. Finally, the SNR values of the four areas are all greater than 0.5dB.

Figure 5A1: the imbibition phase I (0-60h), the SNR value of exocarp showed a sharp rise after a brief rise, then rises quickly, the SNR value of mesocarp and endocarp generally showed a slow upward trend; the imbibition phase II (60h-185h), the SNR values of exocarp experienced two rises and falls, the SNR values of mesocarp and endocarp continued to rise slowly; the saturation stage (185h- 504h), quickly the SNR values of exocarp dropped to saturation and slowly increased after saturation, the endocarp SNR value rises slightly and remains saturated, the exocarp SNR value continued to rise to saturation, and slowly decrease after saturation (504h-744h).

Figure 5A2: the imbibition phase I (0-48h), the SNR value of fruit handle and tip rising sharply; the imbibition phase II (48h-185h), after a sharp decline, the SNR values of fruit handle rises sharply to saturation, the SNR values of tip rise after falling, then fall to saturation, the saturation stage (185h-744h), after the fruit handle and tip SNR values reach saturation, they begin to decrease.

Figure 5B shows the change of SNR data in different areas of the seed during 31d imbibition time. Five areas in the MR images were integrated separately in Figure10A. Figure10A: the seed tail region (black solid square data set), the embryo cavity tail region (red solid circle data set), the shell region (blue solid triangle data set), the embryo cavity tip region (green solid inverted triangle data set), the seed tip region (purple solid rhombus data set).The SNR intensities of the five regions during imbibition are compared as follows: embryo cavity tail > seed tail > embryo cavity tip > seed tip. The SNR data of seed coat was always low, basically maintained at around 0.1dB, showed that the seed coat has almost no water absorption.

Figure 5B 1:the imbibition phase I (0-97h), the seed tail SNR value increased uniformly, the seed tip SNR value increased rapidly; the imbibition phase II (97h-185h), the SNR values of both increased uniformly; the saturation stage (185h- 504h), the SNR values of the seed tail and seed tip both quickly rise to saturation, and slowly decrease after saturation (504h-744h).

Figure 5 B 2: the imbibition phase I (0-60h), the embryo cavity tail SNR value starts to fall and rise, then fall and rise rapidly, the embryo cavity tip SNR value begins to rise and fall, then rise; the imbibition phase II (60h-185h); After falling, the SNR values of embryo cavity tail and tip rises again, then falls again and rises to saturation; the saturation stage (185h- 504h) the embryo cavity tail SNR value rises slowly, the embryo cavity tail SNR value keeps falling. After the SNR values of embryo cavity tail and tip reached saturation, they continuously rising.

Figure 5 C shows the change of SNR in different areas of the embryo in the drupe during 31d imbibition time. Four regions of the embryo MR images are integrated into Figure 4 C respectively: the radicle region (black solid square data set), the cotyledon region (red solid circle data set), the endosperm region (blue solid triangle data set), the shell region (green solid inverted triangle data set). The SNR intensities of the four regions during imbibition are compared as follows: radicle > cotyledon > endosperm > seed coat. All inflation time displayed that the SNR values of the radicle and cotyledon areas are always above 0.6dB, and the SNR values of the endosperm and seed coat areas are always below 0.4dB.

Figure 5 C 1: the imbibition phase I (0-97h), the SNR values of radicle and cotyledon rise sharply and then drop rapidly, and then rise sharply; the imbibition phase II (97h-185h), the SNR values of radicle and cotyledon decreased and then rose, the saturation stage (185h- 504h), the SNR values of radicle and cotyledon continued to rise to saturation, and quickly decrease after saturation (504h-744h).

Figure 5 C 2: the imbibition phase I (0-48h), the SNR values of endosperm and shell are on the rise; the imbibition phase II (48h-161h), the SNR values of endosperm and shell are decreased, rise, fall and rise again; the saturation stage (161h- 504h), the SNR values of endosperm keep falling, the SNR values of shell decline slowly, after the endosperm and shell SNR values reach saturation, they begin to increase. (504h-744h). The SNR values endosperm and shell are always low, the SNR data of the endosperm is maintained between 0.15 dB and 0.4dB, and the SNR data of the shell is always below 0.2dB.

Figure 5 D shows the change of SNR in different areas of the embryo in the seed during 31d imbibition time. Four areas in the MR images were integrated separately in Fig.4 D respectively: the radicle region (black solid square data set), the hypocotyl region (red solid circle data set), the cotyledon region (blue solid triangle data set), the endosperm region (green solid inverted triangle data set). The SNR intensities of the four regions during imbibition are compared as follows: hypocotyl > cotyledon > radicle > endosperm.

Fig.5 D 1: the imbibition phase I (0-48h), the radicle SNR value rises and falls, then rises, the cotyledon SNR value rises after falling, the endosperm SNR value drops rapidly after a rapid rise; the imbibition phase II (48h-185h), after three rises and then dropped mode, the radicle SNR value reached to saturation, the cotyledon SNR value rises after two rising and then falling mode to saturation, the endosperm SNR value rises to saturation after a sharp drop; the saturation stage (185h- 504h), the SNR values of the radicle, cotyledon and endosperm both continue(s) to drop, and quickly increase after saturation (504h-744h).

Fig.5 D 2: the imbibition phase I (0-48h), the hypocotyl SNR value rises after continuous decline, the cotyledon SNR value rises after falling, the endosperm SNR value drops rapidly after a rapid rise; the imbibition phase II (48h-185h); the hypocotyl and cotyledon SNR value after two rising and then falling mode to saturation, the endosperm SNR value rises to saturation after a sharp drop; the saturation stage (185h- 504h), the SNR values of the hypocotyls, cotyledon, and endosperm both continue(s) to drop, and quickly increase after saturation (504h-744h). In different periods of inflation, the SNR data changes of the hypocotyl, cotyledon and radicle are basically the same. The SNR data of the endosperm never exceeds 1.1dB.

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. The tip consists of epidermis, cork layer and cortex. The epidermis is regularly distributed with lenticels and highly lignified. The parenchyma cells of different sizes in the cortex are closely packed. The tip consists of epidermis, cork layer and cortex. The epidermis is regularly distributed with lenticels and highly lignified. The parenchyma cells of different sizes in the cortex are closely packed(Fig.6A). The fruit handle is made up of closely packed thin-walled cells containing green cellulose cell walls(Fig.6B). The epidermis of the exocarp is distributed with lenticels and keratinized(Fig.6C). 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.6D). The shell consists of closely arranged skeletal stone cells and round thick-walled cells of varying sizes, presenting a highly corked red color(Fig.6F).

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.6G). According to the determined experiment by further oil red O fat stain, iodine-potassium iodide starch stain, periodic acid-Schiff polysaccharide stain, we found that the starch content and the fat content were high, but less sugar. The fat has high hydrophobicity, and the accumulation of a large amount in the cells does not increase the osmotic potential of the cells, which hinders the free exchange of water inside and outside the embryo, thereby hindering the material metabolism of the endosperm. In addition, dense endosperm cells create severe mechanical barriers. It can be seen that the permeability problem and mechanical obstacle of the endosperm may be an important reason for the embryo fails to germination.

2.5 Tissue observation in transmission electron microscopy

The hydration of *Sinojackia xylocarpa* Hu seeds may be related to changes in the content of endosperm cells. In order to detect the endosperm cell contents, the ultrastructural characteristics of endosperm cell contents of dry *Sinojackia xylocarpa* Hu seeds were compared by transmission electron microscopy (TEM)[15]. Electron microscope observation shows that the inner wall cells of the endosperm at the radicle position contained a large number of high electron-dense substances and lipid droplets, and their shape and size are different. The volume of a high electron-dense substance is larger than that of lipid

droplets, and the lipid droplets are densely arranged. The high electron density substances in the outer wall cells of endosperm at radicle position are smaller, the lipid droplets are arranged tightly, and high electron-dense substances are also present between the lipid droplets.

Compared with the inner wall cells of the endosperm at the radicle position, the high electron-dense substance of the inner wall of the endosperm in the middle position is larger, accounting for 1/2 of the cell volume, and the lipid droplets are densely arranged. The high-electron dense substance in the outer wall cells of the endosperm in the middle part is small, and the volume is relatively small, the lipid droplets vary in size, arranged neatly, and high electron-dense substances are also present between the lipid droplets.

It can be seen that the endosperm contains a large number of lipid droplets and high electron-dense substances, which may have certain mechanical obstacles to the germination of the embryo.

2.6 Experimental studies on the germination characteristics of the *Sinojackia xylocarpa* Hu seeds and embryos

The germination of the peeled seed, the seeds with partial endosperm, and embryo were 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%. Neither the peeled seeds nor the seeds with partial endosperm germinated. The results of germination experiments fully indicate that the embryos have no dormancy, and the seed coats and the endosperms may cause mechanical obstacles.

3. Discussion

3.1 Peel water permeability of *Sinojackia xylocarpa* Hu

The ultrastructure of the drupe shows that the hardening of the peel begins from tip to the middle of the lignification, the exocarp is keratinized and the hard cuticle forms a protective layer on the surface of the drupe; the surface of the mesocarp is perforated, and the parenchyma cells on the surface are arranged in honeycombs with obvious intercellular space; the cells in the palisade of the endocarp are arranged loosely. These characteristics of the peel can ensure the free flow of water between the exocarp, mesocarp, and endocarp.

3.2 Shell water permeability of *Sinojackia xylocarpa* Hu

The shell is composed of high suberin, fibrously arranged thick-walled tissue with tight cells and no voids. The suberin is a kind of lipid compound in which most of the protoplasts have been disintegrated into dead cells, so the cell wall permeability of the suberin is extremely poor. The seed coat is thick and membranous and waxy. The structure of the seed shell and seed coat plays a role in protecting the embryo, but it may also have mechanical resistance to the germination of the embryo, especially the

growth and elongation of the radicle. In addition, the highly lignified seed coat and dense seed coat structure have a certain hindrance to the free exchange of water inside and outside the seed embryo, which affects the material metabolism process inside the seed.

3.3 Endosperm permeability of *Sinojackia xylocarpa* Hu

The endosperm cells of the *Sinojackia xylocarpa* Hu seed are highly keratinized and densely arranged, and the water is difficult to flow freely inside and outside the endosperm, and the endosperm permeability is poor. The endosperm cell wall is very thick, especially the keratinization of the endosperm cell wall in the radicle and the fibrosis of the inner cell wall, which has a serious mechanical obstacle to the germination of the embryo, so that the radicle cannot break through the resistance of the endosperm. The results of comprehensive scanning electron microscopy, transmission electron microscopy, and paraffin section analysis showed that the mechanical barrier and permeability of endosperm were the important reasons for the inability of embryos to germinate.

4. Conclusions

The moisture entry channel, molecular mobility, and water distribution in *Sinojackia xylocarpa* Hu drupes and seeds during imbibition process, have been monitored through magnetic resonance imaging (MRI), scanning electron microscopy (SEM), transmission electron microscopy (TEM), paraffin section detection (PSD) and germination determination experiment.

We conclude from our MRI experiments that water distribution in *Sinojackia xylocarpa* Hu fruit and seed is inhomogeneous, and fruit tissues hydrate at different rates and extent. The unique feature of *Sinojackia xylocarpa* Hu fruit is the presence of the high-water signal in dry fruit in areas corresponding to vascular bundles. However, the exact explanation of this phenomenon is not known.

The MRI revealed the enter route of water and distribution during imbibition in *Sinojackia xylocarpa* Hu fruit and seed. Water moves initially through the fruit handle hole, then along the vascular bundle and endocarp enters the drupe, next penetrates into the mesocarp and exocarp gradually. After that, the fruit tip begins to enter the water, eventually removing the position of the seed shell, and the entire fruit cavity was filled with water. From the MR images of the drupe and amplified, water begins to enter the radicle and hypocotyl, next permeate to the cotyledon, then enters from the small hole in the lower right end of the endosperm. Finally, the whole embryo filled with water, and the volume of the embryo is gradually enlarged to the entire embryo cavity. The channel of moisture entering was the radicle and the small hole in the lower right side of the endosperm. It can be seen that water entered from the seed tail and the seed tip, water entered from seed tip penetrated into cotyledon, while water entered from seed tail, then moved to the hypocotyl from both the right lower pore of the endosperm and the radicle, then the water gradually fills the seed cavity, and the gap between the seed coat and the endosperm, next the whole seed cavity, and the gap between the seed coat and the endosperm was completely filled with water. But the seed shell always has no water signal. Thus it can be seen the endocarp, and the exocarp was well permeable

and had no permeability barrier. But the hard seed shell and the endosperm have poor water permeability characteristics.

The SEM, TEM, and PSD showed the cell structure characteristics of *Sinojackia xylocarpa* Hu drupe and seed. The fruit handle hole is obvious, the exocarp is keratinized, the mesocarp and endocarp cells are loosely arranged, and the pores are connected in the middle. The seed shell is highly lignified and corky, and the endosperm cells are closely arranged. Consistent with the moisture entry channel and distribution indicated by MRI, the fruit handle hole is the main channel for moisture to enter. The permeability of the endocarp and mesocarp is well, while the permeability and mechanical barrier of the exocarp, seed coat, seed coat, and endosperm are extremely poor. The germination determination experiment indicates that the obstacle of *Sinojackia xylocarpa* seed germination was due to the mechanical restraint of the seed shell and endosperm, and the embryo has no dormancy habit.

From these findings, we can draw the conclusion that the peel has good water permeability but has mechanical obstacles. The hard seed shell caused mechanical obstacles, and the permeability is poor. Neither the peeled seeds nor the seeds with partial endosperm germinated, but the embryos germinated normally. It can be speculated that dense seed coats and endosperms with high-fat content may cause mechanical obstacles. And the endosperm has poor water permeability. It can be seen that mechanical barriers and water permeability problems are the main reasons for the dormancy of the seeds of the *Sinojackia xylocarpa* Hu drupes.

Therefore, in order to break the dormancy of the *Sinojackia xylocarpa* drupe, firstly, the mechanical barrier of the external dense structure should be removed, and then the problem of the endosperm permeability should be removed, which will be verified in the subsequent experiments. Next, we will explore how to reduce the mechanical barriers and permeability barriers of the pericarp, seed shell, seed coat, and endosperm by sulfuric acid treatment, and accelerate the process of dormancy release by low-temperature stratification.

5. 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 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.

5.2 Measurement 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[16]. The tissues taken from different parts of the drupe were fixed on the sample table with double-sided glue[17], then sprayed with a thin gold film on the surface, and observed under the scanning electron microscopy (FEI, American)[18].

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[19]. The drupe and the seed were fixed on the 25×75mm microscope slides in a 45ml centrifuge tube[20]. 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±1°C. 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[21]. 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[22], then observed under an optical microscope (Nikon, Tokyo, Japan)[23].

5.5 Measurement of transmission electron microscopy (TEM)

The transmission electron microscopy was used to analyze the individual cellular components in *Sinojackia xylocarpa* Hu's seeds[24]. The tissues taken from the endosperm were immediately fixed in electron microscopic fixative (Servicebio, Wuhan, China) for 2 hours, then transferred to fixed in 1% OsO₄ for 5 hours, then dehydration through the ethanol series and finally embedded in acetone blocks[25]. The acetone blocks were sliced on a microtome with a glass knife to a thickness of 60nm and stained in 5% uranyl acetate and 0.5% lead citrate, then observed under the transmission electron microscope HT7700 (hitachi, Tokyo, Japan)[26].

Abbreviations

MRI, magnetic resonance imaging; SEM, scanning electron microscopy; TEM transmission electron microscopy; PSD paraffin section detection.

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



Flowers 1



Tree



Flowers 2



Drupes 1



Drupes 2

Figure 1

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

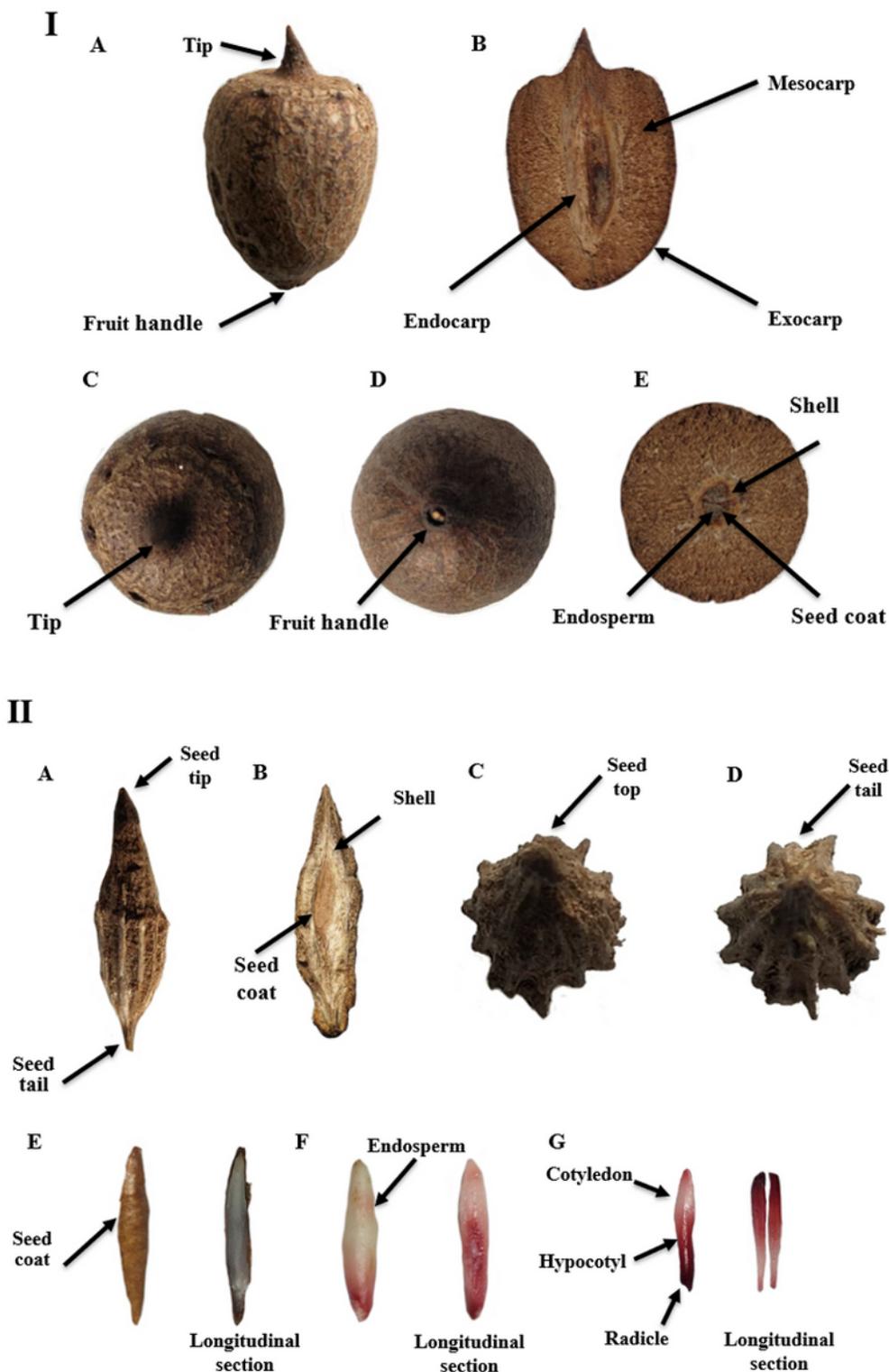


Figure 2

2. The structure of *Sinojackia xylocarpa* Hu drupe. 2. The structure of *Sinojackia xylocarpa* Hu seed. (A) Photograph of seed shape, (B) Longitudinal section anatomy of seed, (C) Photograph of seed top, (D) Photograph of seed tail, (E) Photograph of seed coat, (F) Photograph of endosperm, (G) Photograph of the embryo.



Figure 3

Scanning electron micrographs of the drupe, seed and endosperm surface of *Sinojackia xylocarpa* Hu. (A) Scanning electron micrographs of drupe ,(1)tip outer surface, (2)tip inner surface, (B) Scanning electron micrographs of fruit handle,(3) fruit handle outer surface, (4) fruit handle inner surface, (C) Scanning electron micrographs of exocarp,(5) exocarp outer surface, (6) exocarp inner surface, (D) Scanning electron micrographs of exocarp,(7) exocarp outer surface, (8) exocarp inner surface, (E) Scanning electron micrographs of endocarp,(9) endocarp outer surface, (10) endocarp inner surface, (F) Scanning electron micrographs of out seed coat,(11) out seed coat outer surface, (12) out seed coat inner surface, (G) Scanning electron micrographs of inner seed coat,(13) inner seed coat outer surface, (14) inner seed coat inner surface, (H) Scanning electron micrographs of endosperm,(15) endosperm outer surface, (16) endosperm inner surface.

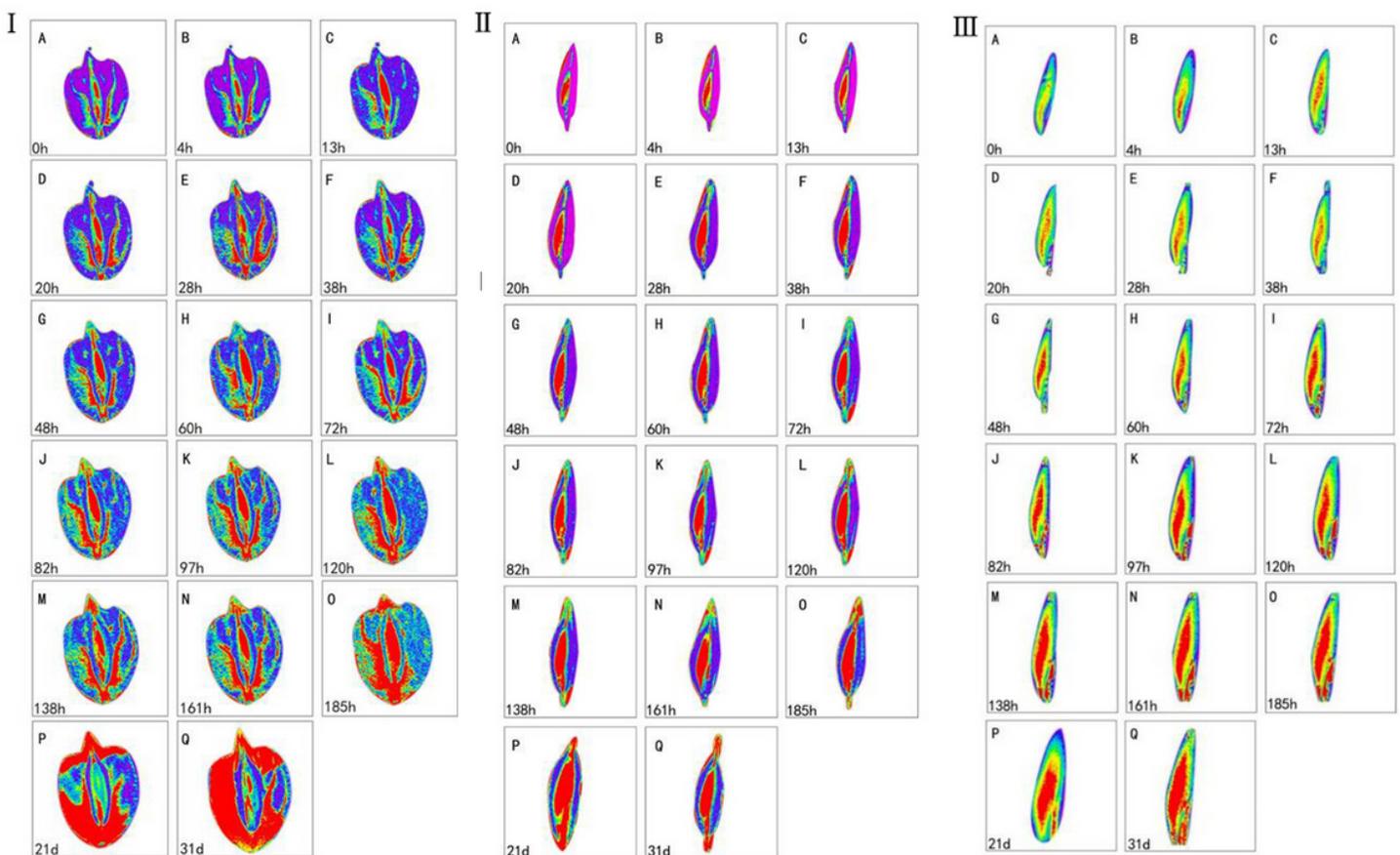


Figure 4

☒. Changes in a *Sinojackia xylocarpa* Hu drupe during imbibition at a median longitudinal section. Images were acquired continuously for 744 h after imbibition, and those presented here are in imbibition for a period of 744 h, as follows: (A) 0h (B) 4h,(C) 13h (D) 20 h (E) 28h (F) 38 h (G) 48h,(H) 60 h (I) 72 h (J) 82h(K) 97 h(L)120 h(M) 138 h(N) 161 h (O) 185 h(P)21d(Q) 31d. The highlighted red signal represents the absorbed water. ☒. Changes in a *Sinojackia xylocarpa* Hu seed during imbibition at a median longitudinal section. Images were acquired continuously for 744 h after imbibition, and those presented

here are in imbibition for a period of 744 h, as follows: (A) 0h (B) 4h,(C) 13h (D) 20 h (E) 28h (F) 38 h (G) 48h,(H) 60 h (I) 72 h (J) 82h(K) 97 h(L)120 h(M) 138 h(N) 161 h (O) 185 h(P)21d(Q) 31d. The highlighted red signal represents the absorbed water. \square . Changes in a Sinojackia xylocarpa Hu embryo during imbibition at a median longitudinal section. Images were taken from the MR images of the embryo, and those presented here are in imbibition for a period of 744 h, as follows: (A) 0h (B) 4h,(C) 13h (D) 20 h (E) 28h (F) 38 h (J) 82h(K) 97h(L)120 h(M) 138 h(N) 161 h (O) 185 h(P)21d(Q)31d.The highlighted red signal represents the absorbed water.

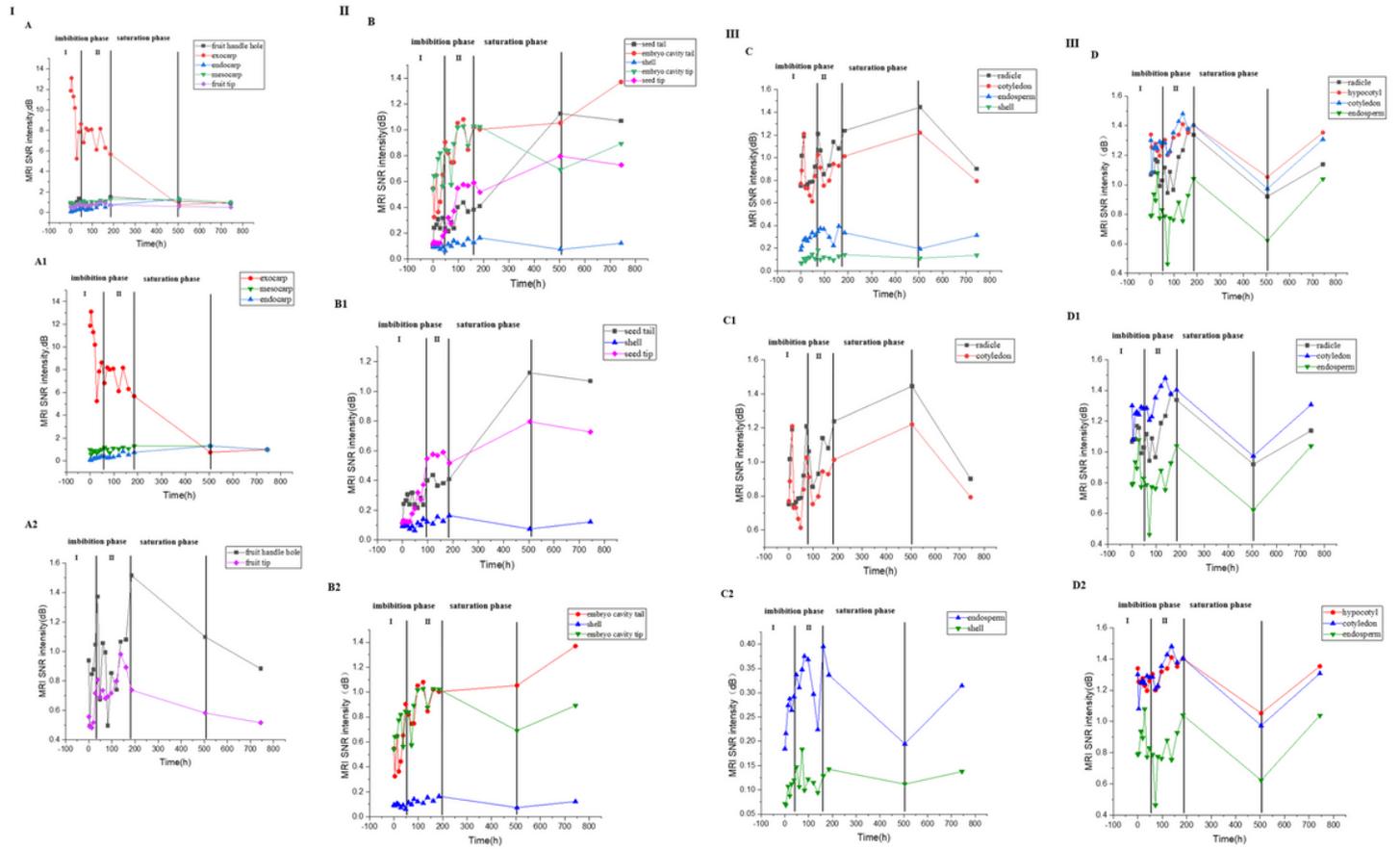


Figure 5

\square . SNR data analyses of water uptake during Sinojackia xylocarpa Hu drupe imbibition. (A) SNR data changes in drupe imbibition during 744h, the fruit handle region (black solid square data set), the exocarp region (red solid circle data set), the mesocarp region (blue solid triangle data set), the endocarp region (green solid inverted triangle data set), and the tip region (purple solid rhombus data set). \square A1 Presentation and comparison of SNR data of exocarp, mesocarp and endocarp during three periods of drupe imbibition \square A2 Presentation and comparison of SNR data of fruit handle hole and fruit tip during three phases of drupe imbibition. \square . SNR data analyses of water uptake during Sinojackia xylocarpa Hu seed imbibition. (B) the seed tail region (black solid square data set), the embryo cavity tail region (red solid circle data set), the shell region (blue solid triangle data set), the embryo cavity tip region (green solid inverted triangle data set), the seed tip region (purple solid rhombus data set). (B1) Presentation and comparison of SNR data of seed tail, shell, and seed tip during three periods of drupe imbibition \square B2 Presentation and comparison of SNR data of embryo cavity tail, shell, and embryo cavity tip during three phases of drupe imbibition.

Presentation and comparison of SNR data of embryo cavity tail, seed coat, and embryo cavity tip during three phases of drupe imbibition. 1. SNR data analyses of water uptake during *Sinojackia xylocarpa* Hu embryo in the drupe imbibition. (C) SNR data changes in embryo imbibition during 744h, the radicle region (black solid square data set), the cotyledon region (red solid circle data set), the endosperm region (blue solid triangle data set), the shell region (green solid inverted triangle data set). (C1) Presentation and comparison of SNR data of radical and cotyledon during three periods of drupe imbibition. (C2) Presentation and comparison of SNR data of endosperm and shell during three phases of drupe imbibition. 2. SNR data analyses of water uptake during *Sinojackia xylocarpa* Hu embryo in the seed imbibition. (D) SNR data changes in embryo imbibition during 744h, the radicle region (black solid square data set), the hypocotyl region (red solid circle data set), the cotyledon region (blue solid triangle data set), the endosperm region (green solid inverted triangle data set). (D1) Presentation and comparison of SNR data of radical, cotyledon and embryo during three periods of drupe imbibition. (D2) Presentation and comparison of SNR data of hypocotyl, cotyledon, and endosperm during three phases of drupe imbibition.



Figure 6

Light micrograph of parenchyma cells in a cross-section through the embryo of *Sinojackia xylocarpa* Hu seed. (A) Parenchyma cells of seed tip, (1) tip whole, (2) tip amplification. (B) Parenchyma cells of seed fruit handle, (3) fruit handle whole, (4) fruit handle amplification. (C) Parenchyma cells of seed exocarp. (D) Parenchyma cells of seed mesocarp. (E) Parenchyma cells of seed endocarp. (F) Parenchyma cells of the seed coat. (G) Endosperm parenchyma cells and inclusions, (5) Endosperm parenchyma cells, (6) Sugar in endosperm, (7) Fat in endosperm, (8) Starch in the endosperm.

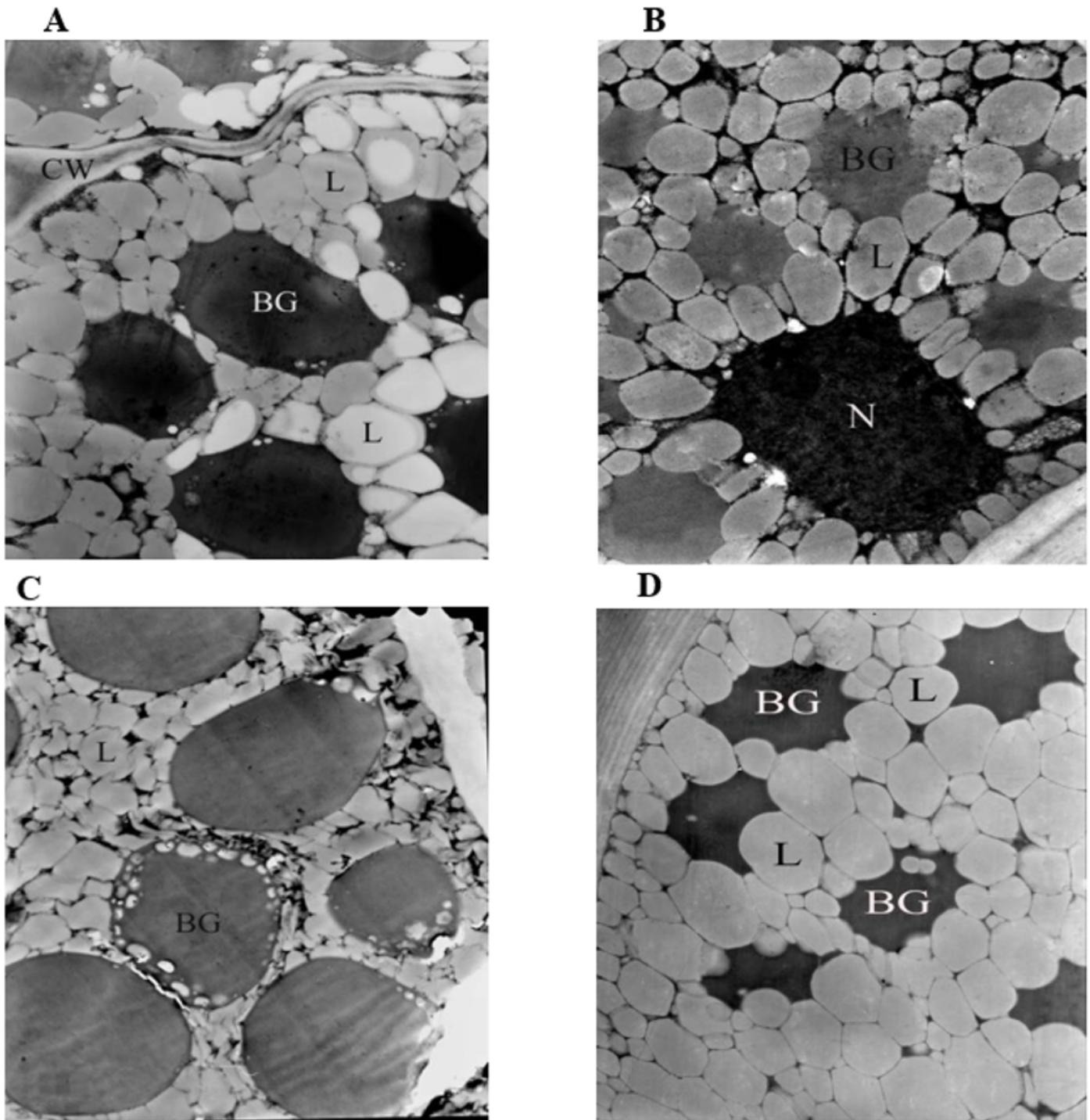


Figure 7

Electron micrograph of endosperm ultrastructure of *Sinojackia xylocarpa* Hu dry seeds. (A) Electron micrograph of the ultrastructure of the inner wall of the endosperm at the radicle position of the dry seed. (B) Electron micrograph of the ultrastructure of the outer wall of the endosperm at the radicle position of the dry seed. (C) Electron micrograph of the ultrastructure of the inner wall of the endosperm in the middle position of the dry seed. (D) Electron micrograph of the ultrastructure of the outer wall of the endosperm in the middle position of the dry seed.

A Germination of peeled seeds



0d



2d



3d

B Germination of embryos



0h



48h



96h

C Germination of seeds with endosperm removed from cotyledons



0d



2d



3d

D Germination of seeds with endosperm removed from radicles



0d



2d



3d

Figure 8

(A) Germination of peeled seeds of 0d,2d,3d. (B) Germination of embryos of 0h,48h, 96h. (C) Germination of seeds with endosperm removed from cotyledons of 0d,2d,3d. (D) Germination of seeds with endosperm removed from radicles of 0d,2d,3d.

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