Discussion on the structure and function of seeds to *Cerasus discoidea*

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**Abstract.** Through morphological anatomy, viability measurement, seed germination and seedling growth observation, it was found that there was a ring band structure between the brown seed coat and embryo of *Cerasus discoidea*, which was the residual endosperm of *Cerasus*, and the seeds of *Cerasus* are composed of seed coat, remaining endosperm and embryo. The remaining endosperm consists of two parts: the endosperm sheath and the endosperm ring. Remaining endosperm is beneficial to the maintenance of seeds viability and the protection of seeds embryo germination and growth.

1. **Introduction**

The drupe is a fleshy fruit developed from a simple pistil or a compound pistil, composed of exocarp, mesocarp, endocarp, and seeds. Both the fruits of Rosacea and *Prunoideae* are drupes. When it matures, it becomes fleshy and does not crack or rarely cracks. Many species are famous fruits and rich in economic value. Most of the seeds are excellent raw materials and can be used as medicine. *Cerasus discoidea* belongs to the *Cerasus* of Prunoideae’s SubFam. The mature fruit is red and can be eaten [1].

According to the record of the general Prunus fruit structure in *Chinese Woody Plant Seeds*, the seeds of *Cerasus* plants are composed of seed coat and embryo[2]; Chu Qinggang et al. dissected the seeds of four *Cerasus* plants and made a statement that the seeds of *Cerasus* plants are composed of seed coat, residual endosperm and embryo [3], but they did not discuss the morphology and function of residual endosperm in detail. In the morphological and anatomical observation of the seeds of *Cerasus discoidea*, the author found that there is a ring-shaped structure between the seed and cotyledons, which is coated on the cotyledon and radicle. This ring-shaped structure has not been described in relevant researches [4-6]. Its developmental sources, structural attribution, etc. were rarely reported. In order to clarify the seed structure of *Cerasus discoidea* and other *Cerasus* plants, this study explored the composition and function of various parts of the *Cerasus discoidea* seed through seed anatomy, viability test, germination and growth observation, so as to provide practical and theoretical basis for the structure and evolution of the seeds of *Cerasus* drupe plants.
2. Materials and Methods

2.1 Morphological and Anatomical Observation of the Seed
Choose a few low-temperature sealed dried *Cerasus discoidea* fruit kernels, break the endocarp and take out the whole seed; soak the seeds in warm water at 25 °C for 24 hours; carry out morphological anatomy and microscopic observation of the *Cerasus discoidea* seeds with tools like surgical blades and tweezers.

2.2 Seed Viability Tetrazolium Determination Test
In order to investigate the composition of *Cerasus discoidea* seeds, and confirm the conclusion of Chuqinggang et al. on the residual ovules of *Cerasus discoidea* plants seeds, we carried out the viability determination test of *Cerasus discoidea* seeds. According to the requirements of forest seed test procedures (GB2772-1999), we chose 100 seeds of *Cerasus discoidea* with endocarp removed randomly, and soaked them in absorbent cotton at 25 °C; after soaking for 18 hours, used tweezers to peel off the brown seed coats, immersed the seeds in the water again for 5 hours, and changed the water once an hour. The seeds were soaked and stained for 12 to 24 hours in the 0.5% Tetrazole solution and in a dark environment of 30-35°C. After the dyed seeds were washed with water, the cotyledons were unfolded on a wet filter paper. Select 100 seeds with seed coats and dye them in the same manner as a comparative test. Repeat three times per treatment. Observe and record the activity of each component of the seed and explore its structural attribution.

2.3 Simulation and Observation of the Germination of Sand Stored Seeds in the Wild State
Select a number of *Cerasus discoidea* kernels that had been sand-stored in low temperature for 90 days and had already cracked, and soil bed sow them in a light incubator; observe and record the process of germination and growth of the *Cerasus discoidea*. During the illumination period, the temperature was 25 °C, the illumination time was 16 hours, the illumination intensity was 750 to 1250 lx; during the dark period, the temperature was 20 °C, and the dark treatment time was 8 hours. This experiment was used to determine the function of the ring-shaped structure between the seed coat and the cotyledon of the *Cerasus discoidea* during seed germination.

3. Results and Analysis

3.1 Seed Structure
The mature *Cerasus discoidea* fruit is red with a kernel inside, which is oval, slightly flat, and the surface has some ribbing. It can be seen from Fig. 1 that the seed of the *Cerasus discoidea* seed with the endocarp removed is elliptical, with a longitudinal diameter of 4.5-6.0 mm and a transverse diameter of about 3.5 mm. The seed coat is yellowish brown and membranous with dark brown longitudinal lines on the surface, and an oval hilum on the side near the radicle end. The hilum has seed holes at one end. There is a ring-shaped structure between the brown seed coat and the embryo, which is coated on the cotyledon. The structure is leathery, hard, milky white, translucent, and flexible. A sheath-like cavity is formed at the lower end of the annulus; the embryo is milky white, composed of cotyledon, germ, hypocotyl and radicle, and a groove is on the back of the cotyledons, which is left by the ring-shaped structure.
Figure 1. The structure of seeds of *Cerasus discoidea* A. seed, endotesta, embryo; B. endotesta sheath; C. longitudinal section in the middle of perpendicular cotyledon surface; D. transection in the middle of seed; E. longitudinal section parallel to cotyledon surface; F. embryo.

The seed generally consists of three parts: seed coat, embryo and endosperm. The seed coat is the protective layer on the outside of the seed. Some plants have only one layer of seed coat, but some plants have two layers of inner and outer seed coats [1]. It was not reported that whether the *Cerasus discoidea* plant has two layers. Chu Qinggang et al. dissected the seeds of four *Cerasus discoidea* plants and they believed that the seed of *Cerasus discoidea* is composed of seed coat, residual...
endosperm and embryo. According to this, the ring-shaped structure might be the endosperm of the *Cerasus*, discoidea plant’s seed, but there is no exact description of the morphology of the residual endosperm. According to the description of the mountain peach’s seeds in *Chinese woody plant seeds*, the seed of such plants is composed of seed coat, cotyledon, germ and radicle, but the ring-shaped structure is not mentioned. There are questions about the source of development of this ring-shaped structure, whether there is viability and structure attribution, and what functions it has.

![Figure 2. Seed viability detection and stained A. embryos stained; B. embryo and endotesta; C. unstaine embryos and stained embryo compared; D. longitudinal section of embryo stained.](image)

### 3.2 Vitality of the Seed Structures

The principle of tetrazolium viability determination is to use a colorless solution of 2,3,5-triphenyl tetrazolium chloride or bromide as an indicator to show the reduction process that occurs in living cells. This indicator is absorbed by the seed and reacts with the reduction process of the living cells in the seed tissue and receives hydrogen from the dehydrogenase. In living cells, 2,3,5-triphenyltetrazolium chloride is hydrogenated and forms a red and unstable diffusion material, that is, triphenyl formazan. This will identify the red living part and the undyed dead part of the seed.

It can be seen from Fig. 2 that cotyledons, germs, hypocotyls and radicles of the vibrant *Cerasus discoidea* embryo are dyed red after the soaking seeds treatment with Tetrazole solution. For some embryos without viability, the cotyledons, germs, hypocotyls and radicles are still milky white after the treatment. The outer ring-shaped structure of the vibrant embryo is also dyed red, indicating that the ring-shaped structure is also vibrant. In the comparative experiment, the brown seed coat of *Cerasus discoidea* seeds were not dyed, indicating that the brown seed coat had died after the seed matured; the inner ring-shaped structure and embryo of the seed coat were not stained, because the
presence of brown seed coat prevented the embryo from being dyed. The *Cerasus discoidea* Tetrazole dyeing vitality determination should be carried out after removing the brown seed coat.

3.3 Seed Germination and Growth

*Cerasus* plants have dormancy, which can be broken by low temperature sand storage. After a certain period of low temperature sand storage, the endocarp of the *Cerasus discoidea* kernel has been cracked. Carry out seeding test on the low temperature sand stored seeds of *Cerasus discoidea* with the endocarp cracked. Through observing the germination and growth change within 1-10 days, it was found (Fig. 3) that after the endocarp on the radicle side of the *Cerasus discoidea* was cracked, the radicle penetrated the sheath-shape cavity and the brown seed coat under the ring-shaped structure outside the embryo, the radicle and the hypocotyl rapidly elongated downward, the upper end of the hypocotyl is bent, and the upper end of the kernel is downward. As the hypocotyl is continuously elongating, the cotyledon is taken out of the soil surface, and during the process, the seed’s ring-shaped structure together with the brown seed coat and the endocarp are separated from the cotyledons, relying on the cotyledon tension, soil resistance and the growth power of the primary leaves. The ring-shaped structure outside the embryo are not absorbed and digested, mainly playing a protective role in seed germination. After coming up out of the ground, due to photosynthesis, cotyledons, hypocotyls, and primary leaves turn green, hypocotyls grow straight up, cotyledons open, primary leaves sprout, and epicotyl elongates, the folded primary leaves gradually open and the seedlings are formed. Thereafter, the radicle and germ respectively develop into the underground root system and the above-ground stem and leaf system.

According to the position of cotyledon at the time of seed germination, the seedlings were divided into epigaeous seedlings and hypogaeous seedlings. From Fig. 3, we can see that the seedlings of the *Cerasus discoidea* are epigaeous seedlings. During the seed period, two primary leaves are folded in the cotyledons. After the seedlings are unearthed, the first pair of primary leaves are growing opposite, and the third true leaf begin to alternate. When the seedlings grow for about 40d, the cotyledons gradually turn yellow and shrink, and begin to fall off after about 70 days. The nutrients stored in the cotyledons are absorbed, and the cotyledons mainly play a role in providing nutrients during seed germination and growth.
Conclusion

The seed coat is a protective layer on the outside of the seed (developed from integument). The number, color and appendage of the seed coat vary depending on the plant species. In the anatomical structure of the *Cerasus discoidea* seed, there is a ring-shaped structure between the brown seed coat and the embryo. The vitality test result shows that the ring structure has vitality, and only the embryo and endosperm in the seed structure have vitality, so according to the early research results of Chu Qinggang et al., this structure should be the residual endosperm of *Cerasus discoidea* seed. During the seed germination, the ring-shaped residual endosperm falls off with the seed coat and endocarp, and does not provide nutrition for the late growth of the seedling, but plays a protection role for the embryo initiates. The author dissected the seeds of *Cerasusserrulata, Cerasusyedoensis, Cerasuscampanulata* etc. of *Cerasus* of Prunoideae’s SubFam, and found that they also had ring-shaped residual endosperm. Since the residual endosperm morphology of the *Cerasus* of Rosaceae has not been reported before, here the author revised the seed structure of the drupe plants of *Cerasus* of Rosaceae. The seed is composed of seed coat, residual endosperm and embryo (Fig. 1), and the residual endosperm is the ring-shaped structure between the seed coat and the embryo, including the endosperm sheath and the endosperm ring. The endosperm sheath is the sheath-shaped structure surrounding the radicle and under the lower end of the endosperm, and the endosperm ring is on the back of the cotyledon of the embryo, leaving a shallow groove on the back of the cotyledon.

The cotyledon of *Cerasus* plants seed is thick, accounting for the most part of the embryo, and only the base of the cotyledons is connected with the hypocotyl. If there is no endosperm, the cotyledon of the seed is easily separated with the hypocotyl during seed’s germination and growth. At the same time, when seeds are germinating, it is necessary to break through the hard endocarp. If there is no hard ring-shaped endosperm, the embryo is susceptible to mechanical damage. Therefore, the existence of residual endosperm and seed coat can effectively protect the embryo and avoid the separation of cotyledon and hypocotyl, and also avoid mechanical damage. In the detection of seed viability, it was
found that the residual endosperm of the vibrant _Cerasus discoidea_ seed was dyed red and had a certain vitality, while the radicle was covered by the vibrant endosperm sheath, and the cotyledon was covered by the endosperm ring, so the residual endosperm can effectively maintain the vitality of the radicle and cotyledon and prolong the life of the seed. From the seed germination and growth test of _Cerasus discoidea_, we can know that the radicle has no function of absorbing nutrients during germination. The nutrients needed for radicle and hypocotyl elongation are mainly supplied by cotyledons. According to this, we can know that the cotyledon is the nutrients storage structure in the seed of _Cerasus discoidea_, providing nutrients for seed germination and seedling’s early growth.

In the evolution of angiosperms, there is always the tendency of the degeneration and disappearance of endosperms, and the volume of embryos increasing continuously. The presence of a relatively large embryo causes the possibility that the embryo itself could accumulate and store material in its cotyledon, which is a significant advance because the nutrients accumulated in the cotyledon are more easily absorbed by the germinated embryos than the endosperm nutrients surrounding the embryo. In the existing seed plants, the embryos which have the absorption and photosynthetic cotyledons are primitive, while the storage cotyledons are evolutionary. At the same time, in the evolution of fruits, the drupe is more advanced compared to other fruit types. Therefore, the seeds of _Cerasus_ plants are more evolved; the existence of residual endosperm can avoid the separation of cotyledons from hypocotyls, protect them from mechanical damage, maintain radicle and cotyledons, and prolong seed life, which is the result of seed evolution of _Cerasus_ plants.

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