Postprint: Morphological and Anatomical Characteristics During Moso Bamboo Seed Formation

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Abstract

To reveal the developmental patterns of embryo, endosperm, pericarp, and seed coat during Phyllostachys edulis seed growth, and to provide a theoretical basis for improving the reproductive biology of moso bamboo and understanding embryo and endosperm development in bamboo plants. Using flowering moso bamboo from the Haiyangshan area of Guilin as material, seeds at different developmental stages were collected and fixed, paraffin sectioning was employed to prepare slides, and structural changes in the embryo, endosperm, pericarp, and seed coat were observed under microscope. The results showed: (1) Fertilization was completed and a zygote formed 1 day after anthesis in moso bamboo, with a zygote dormancy period of approximately 5 days. Subsequently, after passing through the proembryo stage, coleoptile stage, young embryo growth stage, and mature embryo stage, embryo development was essentially mature 40 days after anthesis, with its developmental type being the Gramineae type. (2) Endosperm development preceded embryo development, with its developmental type being nuclear endosperm, undergoing four stages: free nuclei, cellularization, cell differentiation, and maturation. During the cell differentiation stage, endosperm cells differentiated into starchy endosperm cells and aleurone layer cells, with starchy endosperm cells primarily accumulating starch granules and aleurone layer cells primarily accumulating mineral elements, lipids, and proteins. (3) One day after anthesis, pericarp cells and integument cells were regularly shaped, rich in contents, and structurally intact; 10–20 days after anthesis, the number of cell layers in the inner and outer pericarp and integument decreased, their shapes changed, and starch granules began to appear in mesocarp cells; 20–60 days after anthesis, as endosperm cells accumulated nutrients and increased in volume, they exerted outward mechanical pressure, causing mesocarp cells to gradually disintegrate, leaving only residual cell walls; outer pericarp cells were elongated with thickened cell walls, forming a protective structure together with the residual mesocarp cell walls. In moso bamboo, endosperm development precedes embryo development; embryo development undergoes five stages and belongs to the Gramineae type; endosperm development undergoes four stages and belongs to the nuclear endosperm type. The pericarp mainly functions to synthesize and transport nutrients and protect embryo and endosperm development during seed development.

Full Text

Preamble

Morphological and Anatomical Characteristics of Phyllostachys edulis Seeds During Formation Process

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Abstract

This study reveals the developmental patterns of the embryo, endosperm, pericarp, and seed coat during the growth process of *Phyllostachys edulis* seeds, providing a theoretical basis for improving our understanding of reproductive biology in bamboo species and the development of embryos and endosperm in bamboo plants. Using flowering *Phyllostachys edulis* from the Haiyangshan region of Guilin as experimental material, seeds at different developmental stages were collected and fixed. The paraffin sectioning method was employed to prepare samples, and structural changes in the embryo, endosperm, pericarp, and seed coat were observed under a microscope.

The results showed that: (1) Fertilization and zygote formation in *Phyllostachys edulis* were completed within 1 day after flowering, and the zygote entered a dormant period lasting approximately 5 days. The embryo subsequently progressed through the proembryo stage, coleoptile stage, young embryo growth stage, and mature embryo stage, reaching basic maturity 40 days after flowering. The developmental type was categorized as graminaceous. (2) Endosperm development preceded embryo development, following the nuclear endosperm type and proceeding through four stages: free nuclear, cellularization, cell differentiation, and maturation. During the cell differentiation stage, endosperm cells differentiated into starch endosperm cells and aleurone layer cells. Starch endosperm cells primarily accumulated starch granules, while aleurone layer cells mainly accumulated mineral elements, lipids, and proteins. (3) One day after flowering, pericarp cells and integument cells exhibited regular shapes, rich contents, and intact structures. Between 10–20 days after flowering, the number of cell layers in the inner and outer pericarp and integument decreased, cell shapes changed, and starch granules began to appear in middle pericarp cells. From 20–60 days after flowering, as endosperm cells accumulated nutrients and increased in volume, they exerted outward mechanical pressure, causing middle pericarp cells to gradually disintegrate, leaving only residual cell walls. Outer pericarp cells became elongated with thickened cell walls, forming a protective structure together with the remaining middle pericarp cell walls. In summary, endosperm development preceded embryo development in *Phyllostachys edulis*. Embryo development progressed through five stages and was classified as graminaceous, while endosperm development proceeded through four stages and was classified as nuclear-type. The cortex primarily functioned to synthesize and transport nutrients and protect the developing embryo and endosperm throughout seed development.

Keywords: *Phyllostachys edulis* seeds, embryo, endosperm, pericarp, seed coat

Introduction

The fruit type of Poaceae plants is predominantly caryopsis, characterized by indehiscent pericarp that is highly fused with the seed coat and difficult to separate; hence it is commonly referred to as a seed (hereafter collectively termed seed) [?]. As one of the reproductive organs of plants, seeds generally consist of an embryo, endosperm, and cortex. The embryo is critical for seed germination and seedling establishment, while the endosperm provides energy support and nutrients for embryo growth and germination, and the cortex serves as the protective structure [?]. Poaceae seeds possess extremely well-developed endosperm tissue, which occupies approximately 90% of the total seed volume, whereas the embryo occupies only 2–3% [?, ?].

Current research on embryo and endosperm development in Poaceae has primarily focused on traditional agricultural crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), and maize (*Zea mays*). The Wang Zhong research team at Yangzhou University has investigated embryo and endosperm development in these crops under various treatment conditions, analyzing their developmental response mechanisms [?, ?, ?, ?, ?, ?, ?]. Additionally, several studies have examined seed development in forage grasses using paraffin sectioning methods, including *Setaria sphacelata* cv. Narok [?], *Elymus dahuricus* [?], *Eulaliopsis binata* [?], and *Elytrigia repens* [?], establishing foundations for reproductive biology research in these species.

However, research on embryo and endosperm development in bamboo species remains limited due to the rare flowering phenomenon and difficulty in collecting seed materials. Only a few systematic studies exist, such as those by Lin et al. [?] and Wu et al. [?] on *Arundinaria simonii* f. *heterophyllus* and *Chimonobambusa utilis*. Other studies by Huang [?] and Qiao et al. [?] have focused only on mature seeds or early developmental stages, lacking comprehensive analysis of developmental changes throughout the entire process.

Phyllostachys edulis, belonging to the genus *Phyllostachys* in Poaceae, is a large, evergreen, scattered bamboo species with high economic value for both shoot and timber production [?]. It is also the most widely planted and extensively studied bamboo species in China, with in-depth research on biomass [?, ?], gene expression [?, ?], community structure [?, ?], and seed phenotypes [?, ?]. Regarding reproductive biology, Qiao et al. [?] collected *P. edulis* ovaries at early flowering stages and observed fertilization processes and primary endosperm nucleus division, providing preliminary analysis of early seed development but without investigating the entire developmental process. Sun [?] conducted comprehensive studies on floral organ morphology, inflorescence and anther development, pollen morphology, and double fertilization, revealing morphological changes in reproductive organs before and after flowering but with limited focus on embryo and endosperm development during seed formation.

In summary, reproductive biology research in Poaceae has concentrated primarily on traditional crops and easily accessible grasses, while bamboo research remains scarce due to infrequent flowering and material collection challenges. Recently, frequent flowering and seeding of *P. edulis* in the Haiyangshan region of Guilin have provided a valuable research opportunity. This study investigates the development of embryos, endosperm, and cortex in *P. edulis* from Haiyangshan using paraffin sectioning to address three key questions: (1) the developmental characteristics and nutrient supply of *P. edulis* embryos; (2) the developmental characteristics and nutrient supply of *P. edulis* endosperm; and (3) the developmental characteristics of *P. edulis* pericarp and seed coat. The aim is to improve our understanding of *P. edulis* reproductive biology and provide theoretical insights into embryo and endosperm development in bamboo species.

1.1 Study Site Description

The experimental site was located in Guanyang County, northern Guangxi, at 110°45 32 E, 25°16 42 N, with an elevation of 822 m. The region has a mid-subtropical climate characterized by hot summers, cold winters, and distinct seasons, with an average annual temperature of 17.9 °C, average annual precipitation of 1,540.7 mm, and average annual sunshine duration of 1,400.2 hours. The experimental forest was a natural *P. edulis* stand with red soil. Understory vegetation consisted primarily of shade-tolerant grasses and shrubs, including *Miscanthus floridulus*, *Pteris semipinnata*, *Pteridium aquilinum* var. *latiusculum*, *Celtis tetrandra*, and *Poria cocos*.

1.3 Experimental Methods

In May 2022, before flowering, all bamboo stems in the sample plot were measured to determine stand factors including diameter at breast height (DBH), age, plant height, height under branches, and canopy density. The natural *P. edulis* stand contained 52 flowering individuals, all perennial with no current-year or two-year-old bamboo, and an average DBH of 8.3 cm. Flowering began on June 10, 2022, and reached full bloom (flowering area exceeding half of the total area) on June 20, 2022. From full bloom to seed maturity (June 20 to August 20, 2022), three healthy bamboo plants with similar DBH and growth status were selected, bent down, and marked with ink dots and tags to record flowering time. For the first 30 days, 100 naturally pollinated ovaries were collected and fixed daily; for the subsequent 30 days, 100 developing seeds were fixed at 10-day intervals. Samples were cross-sectioned with double-sided blades and fixed in FAA fixative (70% ethanol:glacial acetic acid:formalin = 18:1:1) in glass vials according to tissue type.

Fixed seeds were dehydrated through an ethanol gradient, cleared with 1/2 ethanol + 1/2 xylene and 100% xylene (twice), then placed in 1/2 xylene + 1/2 paraffin solution overnight in a 42 °C oven. The following day, samples were transferred to 60 °C paraffin solution for infiltration, with the paraffin solution replaced every 3–4 hours for three total changes. Samples were then embedded, sectioned, and dewaxed before staining with iron alum-hematoxylin, counterstaining with fast green, and final dehydration, clearing, and mounting in neutral balsam. Observations and photography were performed under a Nikon Eclipse Ni-U microscope.

2.1 Embryo Development in *Phyllostachys edulis* Seeds

Embryo development in *P. edulis* closely resembles that of other Poaceae monocots such as rice, oat (*Avena sativa*), *Chimonobambusa utilis*, and *Arundinaria simonii* f. *heterophyllus*, and can be divided into five stages: zygote stage, proembryo stage, coleoptile stage, young embryo growth stage, and mature stage. One day after flowering, the seed entered the zygote stage, with sperm-egg cell fusion forming the zygote and another sperm cell fusing with polar nuclei to form the primary endosperm nucleus. Following fertilization, the zygote entered a dormant period (see [FIGURE:I]A). The proembryo stage occurred from 5–28 days after flowering. Approximately 5 days after flowering, the zygote underwent its first mitotic division to form a two-celled proembryo with basal and apical cells (see [FIGURE:I]B). Subsequently, the apical cell first divided longitudinally then transversely to form a three-celled proembryo, before dividing in multiple directions to form a multicellular proembryo ([FIGURE:I]C–E). Around 24 days after flowering, the multicellular proembryo continued dividing and differentiating into a spherical embryo (see [FIGURE:I]F), which further divided longitudinally and transversely to form a pyriform embryo by approximately 28 days after flowering (see [FIGURE:I]G).

Approximately 30 days after flowering, the *P. edulis* embryo entered the coleoptile stage, characterized by the differentiation of a depression on one side of the embryo to form the coleoptile primordium (see [FIGURE:I]H). Around 40 days after flowering, the embryo entered the mature stage, with organ differentiation essentially complete, including the scutellum, coleoptile, coleorhiza, epiblast, radicle, and plumule, as well as five fully differentiated leaf primordia (see [FIGURE:I]I). Following morphological differentiation, the embryo accumulated nutrients and underwent physiological maturation before entering dormancy through dehydration (see [FIGURE:I]J). Since no samples were collected between 30–40 days after flowering, sections from this period were unavailable;

however, based on sections from 30 and 40 days after flowering, this period represents the young embryo growth stage, during which the embryo rapidly divided and differentiated to form basic structures including the scutellum, plumule, radicle, and embryonic axis. Throughout embryo development, suspensor cells underwent only a few divisions and gradually disappeared after the embryo reached maturity (40 days after flowering).

Plate I. The embryo development of *Phyllostachys edulis*
A. Zygote (1 day after anthesis); B. Two-celled proembryo (5–6 days after anthesis); C–E. Multicellular proembryo (7–20 days after anthesis); F. Globular embryo (24 days after anthesis); G. Pyriform embryo (28 days after anthesis); H. Coleoptile stage (30 days after anthesis); I. Mature embryo (40 days after anthesis); J. Mature embryo (60 days after anthesis). Z. Zygote; Pr. Primary endosperm nucleus; Tw. Two-celled proembryo; Mu. Multicellular proembryo; Gl. Globular embryo; Py. Pyriform embryo; CO. Coleoptile; Ma. Mature embryo.

2.2 Endosperm Development in *Phyllostachys edulis* Seeds

The endosperm development type in *P. edulis* is consistent with most Poaceae plants, classified as nuclear-type endosperm. Based on Poaceae endosperm development patterns, *P. edulis* endosperm development can be divided into four stages: free nuclear stage, cellularization stage, cell differentiation stage, and maturation stage. From 1–5 days after flowering, the endosperm entered the free nuclear stage, during which the primary endosperm nucleus underwent continuous nuclear division without cell wall formation (see [FIGURE:II]A, B). Due to continuous free nuclear division and enlargement of the central vacuole, free nuclei were pushed to the edges of the embryo sac and distributed along the embryo sac wall, resulting in rapid embryo sac volume increase. Around 6 days after flowering, the endosperm entered the cellularization stage, with cell walls forming around nuclei near the embryo, followed by gradual cell wall formation around nuclei at the embryo sac periphery. As cellularization progressed, endosperm cells divided centripetally toward the chalazal end, and by approximately 20 days after flowering, virtually no free nuclei remained in the embryo sac, marking the completion of cellularization. Small starch granules were observed in some endosperm cells during late cellularization, primarily distributed near nuclei (see [FIGURE:II]B–H).

Approximately 20 days after flowering, the endosperm entered the cell differentiation stage, which concluded around 30 days after flowering. During this period, several layers of endosperm surface cells near the embryo sac wall differentiated into aleurone layer cells, while remaining cells differentiated into starch endosperm cells. Aleurone layer cells were characterized by smaller size, larger nuclei, denser cytoplasm, and less starch compared to starch endosperm cells. Starch endosperm cells began differentiating earlier, initiating differentiation at the end of cellularization and accumulating starch granules that gradually filled entire cells as differentiation progressed (see [FIGURE:II]I–N). Thirty days after flowering, the endosperm entered the maturation stage until seed maturity. During this stage, starch endosperm cells underwent programmed cell death, with cell walls gradually disintegrating and nuclei disappearing. Due to cell wall disintegration and continuous starch granule accumulation, starch granules appeared stacked within starch endosperm cells (see [FIGURE:II]O–T). Aleurone layer cells maintained intact cellular structure with nuclei and cell membranes, tightly adhering to the seed coat and reducing from several layers to a single layer during development. Aleurone layer cells also accumulated nutrients during maturation, observed as small granular structures. Degenerated nucellar cells were present in all stages, gradually disintegrating as seed development progressed, leaving only 4–5 layers of nearly completely degraded nucellar cells by 60 days after flowering.

Plate II. The endosperm development of *Phyllostachys edulis*
A–B. Free nuclear stage (1–5 days after anthesis); C–H. Cellularized endosperm stage (6–20 days after anthesis); I–N. Differentiation period of cell (21–30 days after anthesis); O–T. Mature period (31–60 days after anthesis). Fr. Free nucleus; Ce. Cellularized endosperm cells; Al. Aleurone endosperm cells; En. Starch endosperm cells; S. Starch granule.

2.3 Pericarp and Seed Coat Development in *Phyllostachys edulis* Seeds

Phyllostachys edulis seeds possess both pericarp and seed coat, with the pericarp developing from the ovary wall and the seed coat from the integument. One day after flowering (zygote stage), integument cells comprised 4–18 layers of small, regular quadrilateral cells with prominent nuclei. Pericarp cells were divided into three parts: endocarp, mesocarp, and exocarp. The endocarp, connected to integument cells, consisted of 3–5 layers of small, rectangular cells. The mesocarp comprised parenchyma tissue with highly irregular cells much larger than endocarp, integument, and exocarp cells, possessing prominent nuclei and dense cytoplasm. The exocarp consisted of 4–8 layers of square cells with extremely prominent nuclei (see [FIGURE:III]A, A-1, A-2).

By 10 days after flowering (multicellular proembryo stage), integument cell layers decreased from 4–18 to 4–10 layers without changes in morphology or size. Endocarp layers decreased from 3–5 to 2–4 layers with a trend toward smaller morphology. Mesocarp cells enlarged into irregular shapes, with some containing small starch granules around nuclei. Active cell division was observed in mesocarp cells, with binucleate cells detected. Exocarp layers decreased from 4–8 to 2–6 layers, with cell shapes transitioning from square to rectangular. More exocarp layers were observed at the end distant from the embryo, with fewer layers near the embryo (see [FIGURE:III]B, B-1, B-2). By 20 days after flowering (multicellular proembryo stage), integument layers further decreased from 4–10 to 2–4 layers, becoming rectangular with thick, yellow cell walls (before staining). Endocarp layers decreased from 2–4 to 2–3 layers with rounded morphology. Mesocarp cells enlarged into irregular shapes with more starch granules; cells near vascular bundles differed significantly in morphology, appearing more elongated. Exocarp layers decreased from 2–6 to 2–4 layers (see [FIGURE:III]C, C-1, C-2).

By 30 days after flowering (coleoptile stage), integument cells were mostly reduced to 2 layers (4 layers near vascular bundles). Endocarp remained as a single layer tightly adhering to integument cells. Mesocarp cells gradually ruptured, with cell membranes and walls disintegrating; cells near vascular bundles were compressed and reduced in size, resembling integument and endocarp cells. Exocarp cells became rectangular, more slender than in previous stages, with only outer layer cells possessing nuclei (see [FIGURE:III]D, D-1, D-2). By 40 days after flowering (mature embryo stage), integument cells were reduced to a single layer, with endocarp cells tightly adhering. Mesocarp cells near vascular bundles gradually disintegrated, with only some cells retaining nuclei, while mesocarp distant from vascular bundles disappeared completely, leaving only exocarp, endocarp, and seed coat. Exocarp cells became elongated, further lengthened, and nucleated (see [FIGURE:III]E, E-1, E-2). Between 50–60 days after flowering, contents of endocarp, mesocarp, and exocarp cells near vascular bundles essentially disappeared, leaving only remnants of cutinized cell walls (see [FIGURE:III]F–G-2).

Plate III. Structure of *Phyllostachys edulis* seed cortex
A. Cortical structure on the 1st day after anthesis, A-1 and A-2 are details of cortical structure (same below); B. Cortical structure on the 10th day after anthesis; C. Cortical structure on the 20th day after anthesis; D. Cortical structure on the 30th day after anthesis; E. Cortical structure on the 40th day after anthesis; F. Cortical structure on the 50th day after anthesis; G. Cortical structure on the 60th day after anthesis. Nu. Nucellar tissue; In. Integumental cell; En. Endocarp cells; Me. Mesocarp cells; Ec. Ectocarp cells; S. Starch granule; Pe. Pericarp.

3.1 Embryo Developmental Characteristics and Nutrient Supply

Previous research on *P. edulis* reproductive biology has been limited. Qiao et al. [?] observed only basic aspects such as unilocular ovaries, single ovules, synchronized development of pollen and embryo sac, and fertilization processes, without further investigation of embryo development. Sun [?] anatomically examined mature *P. edulis* seeds and briefly described mature embryo and pericarp structures. This study systematically investigated the embryo development process in *P. edulis*, detailing morphological transformations at different developmental stages. We conclude that *P. edulis* embryogenesis resembles that of rice [?], oat [?], and *Arundinaria simonii* f. *heterophyllus* [?], belonging to the graminaceous type, with mature embryos exhibiting typical Poaceae features including plumule, radicle, embryonic axis, and epiblast. Compared with other Poaceae species, *P. edulis* embryo development exhibits several distinctive characteristics: (1) Following double fertilization, Poaceae zygotes enter a brief dormancy period. *P. edulis* has a relatively long dormancy of approximately 4–5 days, similar to *A. simonii* f. *heterophyllus*, whereas wheat and oat dormancy lasts about 1–2 days, and rice only 8–10 hours. (2) The transition from zygote to embryo formation requires specific developmental time. *P. edulis* requires the longest duration at approximately 40 days, while *A. simonii* f. *heterophyllus* requires about 30 days, and rice and oat require approximately 14 days. (3) A globular proembryo stage appears during *P. edulis* embryo development, similar to rice, oat, *Psathyrostachys huashanica*, and *Chimonobambusa marmorea*. However, *C. utilis* and *A. simonii* f. *heterophyllus* differ from *P. edulis* in lacking a globular proembryo stage. (4) Although *P. edulis*, *C. utilis*, and *A. simonii* f. *heterophyllus* belong to Bambusoideae, they exhibit significant differences in embryo morphology. *C. utilis* embryos are irregular with a large scutellum volume; *A. simonii* f. *heterophyllus* embryos are slender and triangular with a large scutellum volume. Mature *P. edulis* embryos are right-angled or isosceles triangles, similar to *Dendrocalamus sinicus* embryos, but with a smaller scutellum volume. (5) Antipodal cells were observed proliferating rapidly at the zygote stage, migrating from the chalazal end to the embryo sac periphery and disappearing before the first zygotic mitosis. Antipodal cells persisted for approximately 2–3 days, slightly shorter than in *A. simonii* f. *heterophyllus*.

Previous studies suggest that antipodal cells generally disappear before or after fertilization, functioning primarily to transport nutrients from nucellar cells to support embryo sac development [**?**, **?**, **?**, **?**], which aligns with our findings. We propose that post-fertilization nutrient supply to the embryo originates not only from antipodal cells but also from the embryo sac wall, suspensor, and endosperm cells. Research indicates that most angiosperm embryo sac surfaces possess absorptive functions capable of digesting surrounding nucellar and integument cells to supply nutrients for endosperm and embryo development [**?**]. We observed gradual disappearance of nucellar cells during endosperm and embryo development, with endosperm cells gradually filling the nucellus, suggesting that degraded nucellar cells may be digested by the embryo sac surface to provide nutrients. The suspensor connects nucellar tissue with integument cells, suggesting that nutrients required for embryo division and differentiation may be transported through suspensor cells. The absence of suspensor cells after 40 days may result from completed embryo differentiation, allowing nutrient supply directly from endosperm cells. Furthermore, fragmented endosperm cells and endosperm cells retaining only cell walls were observed surrounding embryos at various developmental stages, with only cell wall remnants accumulating as seed development progressed. This suggests that embryos may absorb nutrients from programmed death of endosperm cells through epidermal cells, or that endosperm cells retaining only cell walls may function in transporting nutrients required for embryo development.

3.2 Endosperm Developmental Characteristics and Nutrient Supply

This study determined that *P. edulis* endosperm follows the nuclear-type pattern and develops earlier than the embryo, consistent with previous findings [**?**, **?**, **?**, **?**]. Compared with other Poaceae species such as *A. simonii* f. *heterophyllus* and wheat, *P. edulis* endosperm development differs only in developmental timing, while showing consistent morphological characteristics and nutrient accumulation patterns during cell division and differentiation. *Phyllostachys edulis* endosperm development proceeds through four stages: free nuclear stage, cellularization stage, differentiation stage, and maturation stage. The free nuclear stage (1–5 days after flowering) involves continuous division of the fertilized polar nucleus into endosperm nuclei distributed along the embryo sac wall without cell wall formation. The cellularization stage (6–20 days after flowering) involves cell wall formation around free nuclei, which divide to fill the entire embryo sac while nucellar cells further degrade. The differentiation stage (20–30 days after flowering) involves differentiation into starch endosperm cells and aleurone layer cells, with accumulation of starch and other nutrients. The maturation stage (30–60 days after flowering) involves progressive accumulation of starch granules and programmed cell death in starch endosperm cells, during which cell walls and nuclei gradually disintegrate. Although starch endosperm cell structures essentially disappear, accumulation of starch bodies and proteins continues, consistent with findings by Wang et al. [**?**]. During maturation, aleurone layer cells also accumulate granular nutrients. Studies suggest these granular nutrients in aleurone cells are substances required by non-starch endosperm cells, such as mineral elements metabolized into phytin granules that combine with proteins to form complex aleurone grains, and lipid bodies synthesized from fatty acids and phosphoglycerides [**?**]. Throughout development, aleurone layer cells maintain intact cellular structure, likely because they synthesize key hydrolytic enzymes such as amylases and proteases during seed germination to degrade endosperm storage materials, indicating their crucial synergistic role in nutrient supply during germination [**?**, **?**, **?**].

We propose that nutrient supply during endosperm development may be transported by antipodal cells, embryo sac wall, vascular bundles, degraded nucellar cells, and aleurone layer cells, with primary transport agents varying across developmental stages. During 1–3 days after flowering, antipodal cells and the embryo sac wall were the main transport agents. After antipodal cell disappearance, the embryo sac wall digested nucellar cells to absorb nutrients for endosperm development. Research indicates that due to the developmental pattern of embryos and endosperm, no plasmodesmata or vascular bundle connections exist between them and the ovary, requiring nutrients transported via vascular bundles to reach the embryo and endosperm through apoplastic pathways [**?**, **?**, **?**]. In rice and other species, aleurone layer cells and degraded nucellar cells function as the apoplast. By 20 days after flowering, *P. edulis* endosperm cells entered the differentiation stage, forming aleurone layer cells that, together with degraded nucellar cells, created an apoplast connecting to vascular bundles to receive and transport nutrients.

3.3 Pericarp and Seed Coat Developmental Characteristics

During seed development, the cortex primarily functions in nutrient synthesis and transport and in protecting embryo and endosperm development [**?**]. *Phyllostachys edulis* seeds possess both seed coat and pericarp, with the seed coat developing from integument cells and the pericarp from the ovary wall. Integument cell layers decreased progressively from 4–18 to 1 layer, with cell morphology changing from regular squares to rectangles, cell walls gradually thickening, and contents disappearing until only cell walls remained. The pericarp consists of three parts: endocarp, mesocarp, and exocarp. The endocarp connects to integument cells, decreasing from 3–5 to 1 layer and eventually fusing tightly with integument cells to form the seed coat. The mesocarp comprises parenchyma tissue containing chloroplasts capable of photosynthesis and nutrient synthesis, causing the green color of developing *P. edulis* seeds. The exocarp serves as the primary protective structure with thick cell walls capable of withstanding mechanical pressure from increasing endosperm volume [**?**, **?**, **?**].

During *P. edulis* seed development, endocarp cell layers decreased from 3–5 to 1 layer, with morphology transitioning from rectangular to rounded, tightly adhering to integument cells by 30 days after flowering. Mesocarp cells were numerous, highly irregular, and much larger than endocarp, integument, and exocarp cells. From 1–20 days after flowering, mesocarp cells gradually enlarged and increased in number, synthesizing starch granules and other nutrients to supply the cortex, endosperm, and embryo. By 20 days after flowering, endosperm cells filled the entire embryo sac and entered the nutrient accumulation stage. As endosperm cells accumulated nutrients and increased in volume, they exerted outward mechanical pressure on the pericarp. As a parenchymatous tissue without thick cell walls, the mesocarp bore the greatest mechanical pressure and began to disintegrate. With continued seed development, mesocarp disintegration accelerated, and by 40 days after flowering, mesocarp distant from vascular bundles had essentially disintegrated completely, while mesocarp near vascular bundles persisted. By 50–60 days after flowering at seed maturity, mesocarp cell contents had essentially disappeared, leaving only remnants of cutinized cell walls that formed a protective structure together with the exocarp. During seed development, exocarp cell layers decreased from 4–8 to 1 layer, cell morphology transitioned from square to elongated, cell walls gradually thickened, and contents disintegrated. By 50–60 days after flowering, the exocarp combined with remaining cutinized mesocarp remnants to form the final protective structure.

Phyllostachys edulis pericarp and seed coat development is consistent with that of wheat, rice, *A. simonii* f. *heterophyllus*, and *C. marmorea*, all showing gradual loss of mesocarp cell viability and formation of protective structures with exocarp and seed coat cells as seeds mature and endosperm volume increases.

However, *P. edulis* pericarp development differs from *C. utilis*, as *C. utilis* fruits are berries whose pericarp cells do not disintegrate during seed maturation.

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Note: Figure translations are in progress. See original paper for figures.