Morphological, physiological and biochemical changes in *Magnolia zenii* Cheng seed during development

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Abstract
An understanding of the seed dynamics of endangered plant species, such as *Magnolia zenii* Cheng, is important for successful conservation. This study examined the morphological, physiological and biochemical changes that occur in *M. zenii* Cheng during seed development to determine the appropriate harvest stage. The appearance of the seeds was influenced by the physiological and biochemical changes occurring during the developmental period, during which the colour of the fruits changed from green to red, while that of the seed changed from light brown to dark brown. There was a significant decrease in the seed moisture content from 90 to 120 days after flowering (DAF); however, there was no significant change from 135 to 165 DAF. The seeds from 135 DAF onwards showed developed cotyledonary embryos. The seed viability exceeded 95% from 135 DAF onwards; however, the germination percentage was 0, hypothesising that the seeds of *M. zenii* Cheng might exhibit physiological dormancy under certain conditions of this experiment. There was a significant increase in the fat, soluble sugar and soluble starch content of the seeds while approaching maturity. There were significant changes in malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH) and β-amylase activities in the seeds during the developmental period. At 135–165 DAF, the MDH activity remained stable, whereas that of 6-PGDH reached its maximum, indicating that the seeds underwent vigorous metabolism. The findings of this study provide a theoretical basis for researching seed dormancy and seed harvest time.

1 | INTRODUCTION

*Magnolia zenii* Cheng is a deciduous tree belonging to the subgenus *Magnolia* and family Magnoliaceae and is endemic to China. The existing natural communities are distributed on the northern slope of Baohua Mountain in Jurong City, Jiangsu province (Xu et al., 2001). *M. zenii* Cheng is a precious ornamental garden tree, and its flowers and fruits can be used as medicine. Recent studies have shown that the seed extract of *M. zenii* Cheng has anti-inflammatory and anti-tumour effects (Song et al., 2012, 2013; Song & Li, 2012a, 2012b). However, owing to the limited environment range of *M. zenii* Cheng and interspecific competition, the population of adult and seedlings of *M. zenii* Cheng in the wild is threatened (Chen & Nan, 2016; Jiang et al., 2010; Li et al., 2019).
Wang & Xie, 2004; Yu, 1999). In 1999, *M. zenii* Cheng was listed as a national level II threatened species and listed as a critically endangered species in 2004 (Wang et al., 2008; Xue et al., 2010). As a critically endangered species, *M. zenii* Cheng faces a habitat crisis. Several studies have been performed to identify and examine the reasons for the low population of *M. zenii* Cheng. Qin et al. (2017) examined the population status of *M. zenii* Cheng in China and proposed solutions for the protection and conservation of the species. Wang et al. (2019) suggested that the specificity of *M. zenii* Cheng to certain environmental conditions and its inadaptability could have contributed to its low population. Moreover, other external factors, such as the destruction of the original habitat of *M. zenii* Cheng, may have contributed to the low population. Additionally, the reproductive dynamics of *M. zenii* Cheng, including flowering, pollination, male and female sexual organs and seed setting, may contribute to its low population.

As an original group of angiosperms, most plants of the family Magnoliaceae are endangered species and are sensitive to global climate change, with more than 50% of the known species seriously threatened (Hu et al., 2015). Recently, several research efforts have focused on examining the distribution of Magnoliaceae and identifying factors limiting the population and distribution of its species (Qing et al., 2017). In addition to adverse environmental factors (Han et al., 2020), the population and distribution of plants of the family Magnoliaceae are threatened by internal factors, among which the most important are reproductive barriers. Therefore, it is particularly important to understand the developmental characteristics of the seeds (Buijs et al., 2020; Meier et al., 2019; Oluwole et al., 2020). Owing to the lack of wild resources of *M. zenii* Cheng and artificial cultivation methods, the seeds are difficult to harvest for propagation. Additionally, a large quantity of available seeds is eaten by birds and rats; moreover, the seed germination percentage is low, which affects seedling production and propagation. Therefore, developing an artificial cultivation method for *M. zenii* Cheng is an important approach in solving the problem of seed scarcity. Among artificial breeding methods, seed breeding is the most economical and sustainable approach for growing *M. zenii* Cheng seedlings. However, lack of basic information on the seed developmental process and stages have limited the production of *M. zenii* Cheng seedlings. Therefore, studies on the seed development and maturation process are necessary. Presently, studies on *M. zenii* Cheng are mostly focused on the community distribution characteristics (Chen et al., 2015; Ri-Ming et al., 2000; Wu, 2004; Zhang, 2007). Understanding of the developmental characteristics of the seed, especially the development status of endosperm and seed embryo, the ultrastructure of endosperm and the physiological and biochemical changes during the development process, is still limited.

Therefore, the aim of this study was to examine the morphological, physiological and biochemical changes that occur in *M. zenii* Cheng seed during development. Additionally, we examined the growth characteristics and dormancy of *M. zenii* Cheng seeds.

## MATERIALS AND METHODS

### 2.1 Test materials

Seeds of *M. zenii* Cheng were collected from a more than 10-year-old plantation in Jiangsu Agriculture Park, Biancheng Town, Jurong City, Jiangsu Province, China (119°09′ 58″ E, 31°57′ 50″ N). Five mature plants with robust and good growth were randomly selected for experimental observation. The climate of Jiangsu Agriculture Park is a subtropical monsoon, with abundant water and sunlight. The annual precipitation is 961.0 mm, with significant annual variations. The annual average temperature is 15.4°C.

### 2.2 Experimental design

The first set of samples were collected on the April 20, 2019, 30 days after the flowering stage, and then every 15 days until the seeds were mature and naturally shed. We designed a total of 10 sampling times until the seed fell off. During sampling, 150 fruits were randomly picked from around the tree crown and quickly stored in an icebox for transportation to the laboratory.

### 2.3 Methods

#### 2.3.1 Morphological observation of the seeds

After each sampling, the fruits and seeds of the follicles were observed and photographed using an anatomical lens and cut into cross-sections using a scalpel. Thereafter, the endosperm structure at different developmental stages was photographed for subsequent examination. The embryo development process was recorded by photographing from the stage when the embryo could be observed, and the embryo length was measured by means of an anatomic mirror measurement tool. Thirty seeds were randomly selected using the quartering method and repeated three times. A vernier calliper (0.01 mm accuracy) was used to measure the transverse and longitudinal diameters of the seeds. The dry and fresh weights of the seeds were determined using an electronic balance (0.01 g accuracy).

#### 2.3.2 Determination of seed moisture content

According to the forest seed inspection regulations (GB2772-1999), the moisture content of the seeds was determined using the oven method at 103°C for 17 ± 1 h until a constant weight was achieved, using three replications composed of 50 seeds. The moisture content of the seed was calculated using the formula below:

\[
\text{Water content} = \frac{M2 - M3}{M2 - M1} \times 100\%
\]
where M1 is the dry weight of the sample box and lid; M2 is the weight of the sample box, lid and sample before drying and M3 is the weight of the sample box, lid and sample after drying.

2.3.3 | Determination of seed viability and in vitro embryo culture

Seed viability was determined using tetrazole (TTC) solution staining. Briefly, 50 seeds were randomly selected and soaked in water until full imbibition and repeated four times. The seed shells were removed, and the seeds were cut longitudinally with a single-sided blade to expose the seed embryos without cutting the seed embryos and then immersed in a 0.5% TTC solution for 4 h at 35°C in the dark. The viability of the seeds was interpreted according to GB2772-1999 Tree Seed Inspection Rules. The embryos were placed in a Petri dish covered with wet filter paper and cultured in a light germinating chamber at 25°C.

2.3.4 | Fresh seed germination test

At the stage 135–165 days after flowering (DAF), 50 seeds were randomly selected for germination test in a growth chamber and grown in a germination chamber at 25°C under a 24-h photoperiod for 30 days. The development of a visible cotyledon indicated successful germination. After the 30-day germination period, the number of germinated and ungerminated seeds was determined.

2.3.5 | Preparation of paraffin sections of seeds

After each sampling, the seeds and flower buds were removed from the fixation solution and smoothed in a fume hood. The seeds were then dehydpercentaged through an ethanol series using 75% ethanol for 4 h, 85% ethanol for 2 h, 90% ethanol for 2 h, 95% ethanol for 1 h and anhydrous ethanol for 20 min. Thereafter, the samples were immersed in alcohol-benzene for 10 min, xylene for 10 min and paraffin for 1 h. The samples embedded in paraffin were subsequently cut into 5-μm-thick sections using a microtome. The sections were treated in warm water (35°C) for 20 min, baked in an oven at 60°C for 30 min, and then taken out. The slices were successively put into xylene twice for 20 min, anhydrous ethanol twice for 5 min twice, 75% alcohol for 5 min and washed under running water. After washing, the slices were treated in 50, 70 and 80% alcohol for 8 s, followed by solid green staining for 15 s. After dehydration in three vats of anhydrous ethanol, slices were placed in pure xylene for 5 min. The slices were sealed with neutral gums for microscopic examination, and images were collected.

2.3.6 | Transmission electron microscope observation of seed endosperm structure

The structure of the seed endosperm was examined using a transmission electron microscope. We randomly selected 10 seeds for transmission electron microscopy. The seeds were cut to form cubes of 1 mm³, immersed in a solution containing glutaraldehyde 2.5% in 50 mm phosphate buffer (pH: 7.8) and stored at 4°C in a refrigerator. Thereafter, the samples were rinsed four times for 20 min each, dehydpercentaged using a series of ethanol solution, soaked in glyc-eryl aliphatic epoxy resin, embedded and polymerised, and then cut into sections. Finally, the cells were stained twice. Images were observed using a Hitachi 600 transmission electron microscope (Pannoramic MIDI/250, 3D HISTECH).

2.3.7 | Determination of nutrients

After each sampling, the endosperm of 90 seeds was removed, and the embryos were removed after vertical cutting. The endosperm tissue was chopped and mixed, and 0.3 g was used for physiological examinations. Each physiological parameter was examined three times. Thereafter, the endosperm tissue was stored at −80°C in an ultra-low temperature refrigerator for subsequent analyses. The soluble sugar content of the endosperm was determined using the anthrone colorimetric method (Wang, 2006a), while starch content was determined using the iodine colorimetric method (Xu et al., 1998). Soluble protein content was determined using the Coomassie brilliant blue method (Wang, 2006b), while the crude fat content was determined using the Soxhlet extraction method (GB 2017).

2.3.8 | Amylase activity determination

$\alpha$-Amylase and $\alpha + \beta$-amylase activity was determined using the 5-dinitrosalicic acid (DNS) method (Wang, 2003), and malate dehydrogenase (MDH) activity was determined according to the method of Zhu Guangliang (Zhu, 1990). Briefly, 0.1 g of tissue was accupercentagedly weighed. It was then placed in a test tube, and distilled water added at a ratio of weight (g): volume (ml) = 1:5–10. The homogenate was cen trifuged at 3000g, and the extract was kept at room temperature for 15 min. The extract was agitated once every 5 min to facilitate total extraction. The mixture was centrifuged again at 3000g for 10 min at 18°C. The supernatant was heat-treated with hot distilled water to inactivate $\beta$-amylase. The heat-treated supernatant (0.2 ml) was added to 0.5 ml of 100 mmol L⁻¹ Na-acetate (pH 6.0) containing 10 mmol L⁻¹ CaCl₂. Reaction was initiated with 0.5 ml of 2% (w/v) soluble starch. After incubation at 37°C for 15 min, the reaction was terminated by adding 0.5 ml of 40 mmol L⁻¹ DNS solution containing 400 mmol L⁻¹ NaOH and 1 M K–Na tartrate; it was then placed immediately in a boiling water bath for 5 min. After dilution with distilled water, the A530 of the reaction mixture was measured, and reducing power was evaluated using a standard curve obtained with glucose.
2.3.9 Determination of the activities of key enzymes in the oxidation pathway

The activities of glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) were determined according to the methods of Brown and Wray (1968). Briefly, 0.3 g of fresh endosperm sample was transferred into a test tube, 2 ml of 0.1 mol L$^{-1}$ of Tris buffer (pH 7.4) was added and the mixture was ground at low temperature. The homogenate was transferred into a 10 ml centrifuge tube, centrifuged at 8000g for 15 min at 4°C, and then filtered and centrifuged again for 15 min under the same conditions. The supernatant was stored in a refrigerator at 4°C for the determination of MDH, G-6-PDH and 6-PGDH activities.

2.4 Data analysis

Data obtained during the study were arranged in Excel 2010 and subjected to one-way analysis of variance (ANOVA) using SPSS 23.0, statistical software. Comparison of mean values was performed using Duncan's multiple range test. Mean values were considered statistically significant at $P < 0.01$.

3 RESULTS

3.1 Morphological changes during fruit and seed development

M. zenii Cheng is an aggregate fruit-bearing plant, with each fruit developing about 135–150 carpels and each ovary having two ovules. However, a few ovaries have one ovule. At 30 days after anastomosis, there were visible, green-coloured and straight follicles, consistent with carpel development (Figure 1A1). Additionally, there were two small but fully developed white seeds of equal diameter in each carpel (Figure 1A2). At 45 DAF, the green follicles began to curve, and part of the carpels began to expand (Figure 1B1). The seeds increased in numbers, some of the seeds were very small, with almost no change in diameter, and one or two seeds in some of the carpels have stopped developing even though there was a large seed cavity for continuous growth (Figure 1B2). At 60 DAF, there was a change in the colour of the follicles from dark green to light green, and some seeds stopped developing, resulting in increased curling of the follicles (Figure 1C1); however, the colour of the normally developing seeds had changed from white to light yellowish green (Figure 1C2). At 75 DAF, most of the fruits were light green, while a few fruits had reddish epidermis and continued to increase in volume (Figure 1D1). The aril was light green, and the hilum turned white. When the seed was cut with a knife, the seeds were slightly hard, and the seed coat was light brown and white (Figure 1E3). At 90 DAF, part of the pericarp began to turn pale pink with no obvious change in volume, lignified and hard (Figure 1E1). A small part of the fruit turned red, and the seed coat was white. After the pseudo coat was removed, the seed coat was light brown and hard. At 105 DAF, the colour of the fruits had become reddish, and the seed was very hard; the pseudo seed coat was pink (Figure 1F2). The seed coat was yellow and hard, and the seed coat of most seeds began to turn dark brown near the seed umbilical site (Figure 1F3). At 120 DAF, the fruit had become red, and the pseudo seed coat was orange-yellow, while the colour of the seed coat had changed to dark brown (Figure 1H3). From 135 to 165 days, there was an increase in the intensity of the red pericarp; however, there was no change in the morphology of the fruits (Figure 1H1, I1, J1). The pseudo seed coat colour changed to deep orange (Figure 1H2, I2, J2), and the seed coat changed from dark brown to black, hard and brittle (Figure 1H3, I3, J3). At 165 DAF, the seeds in the fruits began to fall off. Most of the seeds in the fruit were aborted. The fruits seemed to be abundant, but the amount of healthy and full seeds collected was very small.

3.2 Variation of horizontal diameter, vertical diameter, dry weight and fresh weight during seed development

The transverse and longitudinal diameter and fresh and dry weight of M. zenii Cheng seeds are shown in Table 1 and Figure 2. Overall, the parameters were significantly affected by age. There was a significant increase in the transverse and longitudinal diameter of the seeds from 30 to 105 DAF. Thereafter, there was a slight decrease, and the values remained the same up to 165 DAF. There was a significant increase in the fresh weights of the seeds up to 75 DAF, after which it decreased. However, there was no trend in fresh weight from 90 to 165 DAF. Contrarily, the dry weight of the seeds showed an increasing trend, with the highest value recorded at 165 DAF.

3.3 Changes in water content during seed development

As shown in Figure 3, the was a significant decrease in the moisture content of the seeds from 90 to 135 DAF. However, there was no significant difference in the moisture content from 135 to 165 DAF. The moisture content of the seeds at 135, 150 and 165 DAF was 20.79, 20.77 and 20.51%, respectively.

3.4 Morphological changes of the embryo during seed development

As shown in Figure 4, at 90 DAF, transparent, spherical embryos (SE) or heart-shaped embryos (HE) were observed at the other end of the hilum (Figure 4A). At 105 DAF, the endosperm changed from translucent to yellow-green, with a gradually dense texture, and the shape changed from spherical or heart-shaped to torpedo (Figure 4B). At 120 DAF, there was an initial appearance of two closely attached, small cotyledons, and the endosperm was milky white and compact (Figure 4C). The cotyledon was short, the embryo was fully developed, pale yellow, the embryo cavity was obvious and the embryo length
was 1.66 mm (Table 2). From 135 to 165 DAF, the embryo gradually changed from yellow-green to white, and the two cotyledons increased in size (Figure 4D). The embryos at 135, 150 and 165 DAF were 1.82, 1.93 and 1.88 mm, respectively. The embryo rate at 135, 150, and 165 DAF was 0.29, 0.30 and 0.31%, respectively. The differences in embryo length and embryo percentage were not significant, but the embryo cavity was obvious, and the embryo and endosperm were easily separa
centaged.

### 3.5 Seed viability and germination characteristics

The seed viability was determined using the TTC method. Figure 5A–G indicates the seed viability at different developmental stages, and the dye intensity was an indication of the energy consumption of the seedlings. At 90 DAF, the embryo did not retain any dye, indicating that it lacked viability (Figure 5A). At 120 DAF, most of the embryos did not retain dye, indicating that the seed viability was still low (Figure 5B). At 135 DAF, the embryo retained the dye at low intensity (Figure 5C). However, at 150 DAF, the dye retention was high, with deeper intensity at most part of the seed (Figure 5D). At 165 DAF, the dye retention was higher, with deeper intensity in most parts of the seed (Figure 5E). At 135, 150 and 165 DAF, the seed viability was 96, 97.5 and 98%, respectively (Figure 5F, G, H). As can be seen from Table 2, the germination percentage of the seeds at stage 135–165 DAF was 0 even after 15 days of culture, hypothesising that the seeds of *M. zenii* Cheng might exhibit physiological dormancy under certain conditions of this experiment.

### 3.6 Changes in endosperm morphology and ultrastructure during seed development

There was a significant change in the ultrastructure of the endosperm of *M. zenii* Cheng during the developmental period (Figure 6). At 30 DAF, the seeds were yet to form an endosperm, and the seed cells were large, with central nuclei and prominent nucleoli. A large number of plastids, endoplasmic reticulum, Golgi bodies and mitochondria

### TABLE 1 Changes in embryo length with the developmental process of *Magnolia zenii* Cheng

| Days after flowering (days) | Embryo length (mm) | Embryo rate (%) | Embryo morphology characteristics                           |
|----------------------------|--------------------|-----------------|-------------------------------------------------------------|
| 90                         | 0.48               | 0.06            | Spherical or heart-shaped, yellow-green, transparent        |
| 105                        | 1.21               | 0.19            | Torpedo type, yellow-green, transparent                    |
| 120                        | 1.66               | 0.25            | Initial stage of cotyledon embryo, small cotyledons, two closed cotyledons, chartreuse |
| 135                        | 1.82               | 0.29            | Cotyledon embryo, large cotyledons, two open cotyledons, white |
| 150                        | 1.93               | 0.30            | Cotyledon embryo, large cotyledons, two open cotyledons, white |
| 165                        | 1.88               | 0.31            | Cotyledon embryo, large cotyledons, two open cotyledons, white |

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**FIGURE 1** Morphological changes of fruit and seed of *Magnolia zenii* Cheng during its development. A1. Fruit at 30 days after flowering (DAF); B1. Fruit at 45 DAF; C1. Fruit at 60 DAF; D1. Fruit 75 DAF; E1. Fruit 90 DAF; F1. Fruit at 105 DAF; G1. Fruit 120 DAF; H1. Fruit 135 DAF; I1. Fruit 150 DAF; J1. Fruit at 165 DAF. A2. Seed with aril 30 DAF; B2. Seed with aril at 45 DAF; C2. Seed with aril 60 DAF; D2. Seed with aril 75 DAF; E2. Seed with aril at 90 DAF; F2. Seed with aril 105 DAF; G2. Seed with aril 120 DAF; H2. Seed with aril 135 DAF; I2. Seed with aril 150 DAF; J2. Seed with aril at 165 DAF. E3. Seed at 90 DAF; F3. Seed at 105 DAF; G3. Seed 120 DAF; H3. Seed 135 DAF; I3. Seed 150 DAF; J3. Seed 165 DAF.
were distributed in the cytoplasm. The ribosomes were clustered into a large number of multiribosomes and dispersed in the cytoplasm. There were some black osmiophilic drops and starch grains of different sizes distributed in the cytoplasm (Figure 6A1). At the stage 45–75 DAF, there was a gradual increase in seed endosperm area, with transparent gel, and an increase in cell vacuoles, which occupied almost the entire cell (Figure 6B, C, D). The was a decrease in the size of the cytoplasm and the nucleus, and the cell wall was visible. There was also a decrease in organelle numbers, the presence of starch grains and mitochondria activity (Figure 6B1, C1, D1). At the stage 90–120 DAF, a prominent endosperm with dense texture developed, which occupied the entire seed. During this period, the colour of the

**FIGURE 2** Variation of transverse diameter, vertical diameter, dry weight and fresh weight during seed development of *Magnolia zenii* Cheng

**FIGURE 3** Changes of seed water content during seed development of *Magnolia zenii* Cheng
endosperm changed from transparent to white. There was a decrease in the vacuole size. There were some black particles distributed in the endoplasmic reticulum and the edge of the cell wall. There was an increase in the presence of protein bodies and lipid droplets in the cell (Figure 6E1, F1, G1). There was the presence of a spheroid in the seed cells at 120 DAF (Figure 6G). At the stage 135–165 DAF, there was a disintegration of the melanosomes in the seed cells; however, the percentage was slower in some cells. Additionally, there was an increase in the presence of protein bodies and fat droplets in the cells. The lipid droplets were round, oval or irregular in shape and surrounded by the black protein bodies (Figure 6E1, H1, I1, J1). At 150 DAF, the lipid droplets were densely packed in the cells of the endosperm, and the black particles that appeared in the earlier stage could hardly be observed. Single or several aggregated spheres could be observed in the gap between lipid droplets (Figure 6H) and a small amount of black matter gathered around the spheres (Figure 6G). Additionally, the number of spheres in the cells gradually increased until the seeds matured and fell off (Figure 6I, J). The spheres (Figure 6H, I, J) increased gradually from 135 DAF.

3.7 | Nutrient metabolism during seed development

There was a significant increase in the soluble sugar, soluble protein, starch and fat content of the seeds during the developmental period (Figure 7). The soluble sugar, protein and lipid content increased significantly from 30 to 165 DAF, with the highest value recorded at 165 DAF. However, the soluble starch content peaked at 150 DAF, with a slight decrease at 150 DAF. The soluble sugar, protein, lipid and starch content varied in the ranges of 15.3–77.3, 1.6–9.4, 8.5–404.1 and 0.7–15.4 mg g\(^{-1}\), respectively, during the growth period.

3.8 | Changes in activities of amylase and key enzymes in the oxidation pathway during seed development

The \(\alpha\)-amylase activity remained low and almost unchanged during the entire seed development period (Figure 8). However, \(\alpha + \beta\)-amylase...
activity was consistently higher than $\alpha$-amylase activity throughout the growth period. The activity of $\beta$-amylase decreased slightly from 30 to 45 DAF and remained stable afterwards. From 90 to 135 DAF, which is the stage of rapid embryo development, there was a significant increase in the activity of $\beta$-amylase, with the highest value (10.725 U g$^{-1}$) obtained at 135 DAF. MDH is an important enzyme in the TCA cycle, and changes in MDH activity can reflect the occurrence of the TCA cycle and the activity of respiratory metabolism in the seeds. Additionally, G-6-PDH and 6-PGDH are the key enzymes in the pentose phosphate pathway. Overall, the activities of MDH, G-6-PDH and 6-PGDH changed significantly during the growth period, with no obvious trend. However, the activities of G-6-PDH peaked at 135 DAF, which coincided with the period of sugar transformation to high storage compounds.

4 | DISCUSSION

Seed quality, growth characteristics, environmental preference and biochemical properties play an important role in plant conservation. Seed size is closely related to seed development, dormancy, germination and seed longevity. However, seed yield and quality are closely related to seed harvest time. Studies have suggested that seed morphological indexes, physiological and biochemical indexes can be used to determine the appropriate seed harvesting time (Hernández et al., 2020). For example, the moisture content of soybean seeds can be used as an accupercenage index of physiological harvest time (Sun et al., 2020). Additionally, seed colour changes could be used as an indicator of seed maturity; a seed colour change to black is an accupercenage and rapid index of harvest time (Li et al., 2020). In the present study, the were rapid changes in the colour and size of M. zenii Cheng seeds at early developmental stages; however, these indices remained relatively the same at the later developmental stages. The morphological changes of seeds are closely related to the embryo development. A matured M. zenii Cheng seed is characterised by a black or brown colour, with well-developed embryos and relatively constant moisture content. This relationship is important in understanding the internal development of the seeds based on external characteristics.

Seed development is basically the maturation of seeds from fertilisation until they acquire germination status, with well-developed embryos. This process involves several morphological, physiological

**FIGURE 5** (A–L) Determination of seed viability and germination percentage of *Magnolia zenii* Cheng
and biochemical changes. During seed formation, there is an increase in cell size and the accumulation of storage substances such as starch, egg endoplasm, and fat in the embryo and endosperm, which is necessary for germination (Rinne et al., 2001). Knowledge of the physiological and morphological changes that occur during seed development is important for successful seed breeding and seedling production (Song & Choi, 2019). In the present study, there was an increased accumulation of starch and protein in the cells in the early developmental (Krishnan et al., 2020) up to 135 DAF, when the protein content began to reduce. At this stage, the embryo was fully developed and had acquired germination status. The protein and starch reserves were used for embryo development in the seeds. Furthermore, a spherical ball filled with dark was observed in the endosperm cell during this period; however, the role of the spherical ball is not understood. It is speculated that the ball may be linked to the dissolution of the protein bodies during seed maturation (Rashid et al., 2020; Veiskarami et al., 2018; Xia et al., 2017). During this period, there were no significant changes in the morphology, biochemistry and physiology of the seeds (static phase). The role of the spherical ball in the later development process of the seeds of *Magnolia zenii* Cheng needs to be studied further. Wang and Fang (2007) observed that the rapid increase in lipid substances in the endosperm was related to difficulty in the germination of *Cyclophilus sinensis* seed. He suggested that seed dormancy could be broken only after the lipid droplets and highly electron-dense substances were completely degraded. Jia (2008) also reached a similar conclusion in the seeds of *Sinojackia dolichocarpa*.

In the present study, the fat content of *M. zenii* Cheng seed was more than 50%. The period between 30 and 90 DAF is characterised by proliferation of cell division, rapid enlargement of the ovule, rapid increase in endosperm content and gradual increase in soluble sugar and starch accumulation. The period between 90 and 135 DAF is characterised by a rapid increase in soluble sugar, soluble protein and crude fat content and an increase in the number of protein bodies, which was consistent with changes in the physiological parameters examined. Contrarily, there was a rapid decrease in soluble sugar, soluble protein and crude fat content of the seed at the stage 120–135 DAF, which coincided with the period of embryo development. The nutrients were utilised for embryonic tissue differentiation and development (Yang et al., 2011). The plant utilise stored starch to meet the carbon need in cases of scarcity or fluctuations (Wang & Fang, 2007). During the period from the completion of embryo morphological development to the natural shedding of seeds, there was an increase in the accumulation of soluble sugar and soluble starch in the seeds; however, there was no increase in the accumulation of soluble protein and crude fat. The appearance and disappearance of macromolecular substances in the seed endosperm are closely related to seed dormancy and storage.
development (Jia, 2008). Therefore, an understanding of the characteristics of seed development and growth will help in identifying the occurrence of these macromolecular substances.

Since there are several metabolic respiration pathways in plants, changes in the activities of key enzymes in the metabolic pathways can reflect the metabolic activities in plants (Hiroyuki, 2019). Carbohydrate percentage metabolism in the glycolytic pathway is the first step in plant respiration (Zhang, 2008). In the early developmental stages of *M. zenii* Cheng seeds, there is an increase in the metabolic synthesis of sugar and starch to meet seed growth requirements in the short term. Similarly, there was an increase in the activities of β-amylase, but no change in the activities of α-amylase, indicating the β-amylase may play a significant role in the development of *M. zenii* Cheng seed. A large quantity of energy is utilised via the ATP, TCA and MDH activity peaked in the periods of rapid development. Pu et al. pointed out that the seeds of dormant zoysia could not germinate because the intensity of the TCA cycle decreased at the later stage of germination and there was not enough ATP synthesis in the seeds; therefore, the energy needed for cellular metabolic reactions was mainly derived from anaerobic respiration (EMP) (Pu et al., 1996). At the middle stage of seed development, β-amylase activity decreased slightly, and MDH activity increased slowly, while G-6-PDH and 6-PGDH enzyme activities fluctuated, and the PPP pathway was activated (Ribeiro & Costa, 2015). PPP pathway is often closely related to seed development and is an alternate pathway for energy generation (Angelovic et al., 2010). Sugars can be directly oxidised without the EMP pathway to provide raw materials for cell biomolecule construction and nucleic acid synthesis and producing a
large amount of NADPH (Finch & Leubner, 2006; Okamoto et al., 2010). At the later stage of seed development, the PPP pathway in seeds was inhibited, the activities of G-6-PDH and 6-PGDH enzymes decreased rapidly from the highest to the lowest, and the physiological activities of seeds reduced. The failure of seeds to germinate was linked to this change. With the increased activity of G-6-PDH and the increasing proportion of the PPP pathway in glucose metabolism, the seeds of *Panax quinquefolius* (Zhao et al., 2001) and *Euscaphis konishii* Hayata (Zhang et al., 2015) shifted from dormancy to germination, which was also consistent with the results of our study.

Seed maturation is not just a mere standard process, as many researchers believed when studying crop species. Seed maturation is an extremely complex process in which a lot of factors are involved: temperature, water availability, relative humidity, precipitation, among others. The seeds of *M. zenii* Cheng might exhibit physiological dormancy under certain conditions of this experiment. Harvesting seeds is important for propagation purposes and for the conservation of germplasm resources. Our study provides a theoretical basis for researching seed dormancy and seed harvest time. These aspects are highly important for artificial breeding and can not only improve the seed germination percentage but also help us understand the optimal seed storage conditions, to speed up the process.

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**AUTHOR CONTRIBUTIONS**

Shan Wang and Yongbao Shen participated in the discussion and experimental design; Huapeng Bao prepared the experimental materials; Shan Wang performed laboratory analyses and drafted the manuscript. All authors read and approved the final manuscript.

**DATA AVAILABILITY STATEMENT**

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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