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# Kinetics of seed reserve compounds during the maturation of herbaceous peony (*Paeonia lactiflora* Pall.) seeds

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**ABSTRACT:** Seeds of many peony species contain a large amount of oil. However, the exploiting of its potential for oil production is hampered by a lack of basic information regarding the developmental biology of the seeds. Our aim was to obtain a detailed relationship between seed development and accumulation of various storage compounds of *Paeonia lactiflora* 'Hangshao' seed. Seeds were collected at five developmental stages including 30 days after flowering (DAF), 45 DAF, 60 DAF, 75 DAF and 90 DAF. Anatomical and histological analysis, seed weight and water content, proteins, starch, and fatty acids contents were determinated. The time span of seed development for *P. lactiflora* 'Hangshao' was 90 DAF. Seeds were physiologically mature by 75 DAF, with maximum dry matter content. During seed development, the starch and lipid content showed an increased and then decrease pattern, while they reached their maximum content differed with starch for 60 DAF and lipid for 75 DAF. Protein content showed a slight decreased and then increased pattern. Lipid was the main storage reserve of mature seeds. A total of 26 kinds of fatty acid were detected and among which, seven kinds was all more than 100 mg.Kg$^{-1}$ in all developmental seeds. Those seven fatty acids were palmitic acid, stearic acid, oleic acid, trans-oleic acid, linoleic acid, α-linolenic acid, and erucic acid. Besides, the content of α-linolenic acid accounted for more than 40% of the total fatty acid content in each stage.

Index terms: α-linolenic acid, fatty acid, herbaceous peony, histochemistry, seed reserve accumulation.

**RESUMO:** Sementes de muitas espécies de peônia contêm grande conteúdo de lipídios. No entanto, a exploração de seu potencial para a produção de óleo é dificultada pela falta de informações básicas sobre a biologia do desenvolvimento das sementes. Objetivou-se determinar o acúmulo de vários compostos de reserva durante o desenvolvimento de sementes da espécie *Paeonia lactiflora* 'Hangshao'. As sementes foram coletadas em cinco estádios de desenvolvimento: 30 dias após a floração (DAF), 45 DAF, 60 DAF, 75 DAF e 90 DAF. Foram realizadas análises anatômicas e histológicas e o peso de sementes, conteúdo de água, proteínas, amido e ácidos graxos foram determinados. O período de tempo para o completo desenvolvimento das sementes foi de 90 DAF. As sementes estavam fisiologicamente maduras aos 75 DAF. Durante o desenvolvimento das sementes, o conteúdo de amido e de lipídio aumentaram para, em seguida, diminuir, atingindo o conteúdo máximo aos 60 DAF e aos 75 DAF, respectivamente. O teor de proteínas apresentou uma ligeira diminuição e, em seguida, aumentou. Lipídio foi o principal composto de reserva presente nas sementes maduras. Um total de 26 tipos de ácidos graxos foram detectados entre os quais, sete tipos atingiram mais de 100 mg.Kg$^{-1}$ em sementes de todos os estádios de desenvolvimento. Esses sete ácidos graxos foram ácido palmítico, ácido esteárico, ácido oleico, ácido trans-oleico, ácido linoleico, ácido α-linolenic e ácido erécico. Além disso, o teor de ácido α-linolenic representou mais de 40% do teor total de ácidos graxos em cada estádio.

Palavras-chave: ácido α-linolênico, ácido graxo, peônia herbácea, histoquímica, acúmulo de sementes.

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## INTRODUCTION

In the life cycle of angiosperms, seed development is a pivotal and complicated process that connecting two distinct sporophytic generations (Hamamura et al., 2012). This process is usually initiated by double fertilization, where a sperm cell will fertilize a haploid egg and another sperm cell will fertilize a homodiploid central cell in the ovule. This process will lead to the production of a diploid embryo and triploid endosperm (Chaudhury et al., 2001; Hamamura et al., 2012; Gehring and Satyaki, 2017). Many storage compounds are accumulated as seeds develop, including carbohydrates, proteins, and lipids that seeds serve as resources to sustain initial seedling development (Bewley et al., 2013; Zhao et al., 2015). These plant reserves also provide about 70% of calories consumed by human worldwide. The understanding of seed development is of major economic importance as it can be a key factor for the improvement of seed yield and nutritive values increase (Chaudhury et al., 2001; Baud et al., 2008; Liu et al., 2013).

Herbaceous peony (*Paeonia lactiflora* Pall.) is among the most popular garden plants and crowned as the 'prime minister of flowers'. Besides its ornamental value, the dried roots of many herbaceous peony cultivars contain antipyretic and anticonvulsant agents that have been used in traditional Chinese medicine 'Radix Paeoniae Alba' and 'Radix Paeoniae Rubra' for convulsions and analgesic uses (Hong et al., 2001; Wang and Zhang, 2005; Meng et al., 2017). Seeds of peony species have also been proposed as a source of raw material for edible oil (Ministry of Health of the People's Republic of China, 2011; Ning et al., 2015). Seeds of various species and varieties of peony contain 24.0–37.8% oil; > 90% of the fatty acids are unsaturated, such as alpha-linolenic. These fatty acids can provide humans with several health benefits, such as lowering blood pressure, inhibiting platelet aggregation during blood clotting and reducing the overall risk for cardiovascular diseases (Barceló-Coblijn and Murphy, 2009; Zhang et al., 2017). However, the tapping of its potential for oil production is hampered by a lack of basic information regarding the developmental biology of the seeds. For example, very little is known about the relationship between the development of the seeds and the accumulation of nutrient reserves, especially fatty acids.

The focus of our study was *Paeonia lactiflora* 'Hangshao', one of the main cultivars used for medical propose. This cultivar produces a large number of seeds each year (Meng et al., 2018). However, only a small number of seeds are used for propagation (Choi et al., 2009), and most of them are abandoned, which cause a huge waste of resources. Our previous study showed that the seed of *P. lactiflora* 'Hangshao' was abundant in lipid content, about 30% which contained more than 90% unsaturated fatty acid (Ning et al., 2015). However, until now, little is known about the kinetics of storage reserves, especially for unsaturated fatty acid accumulated during seed development.

Our aim was to obtain a detailed picture of seed development of *P. lactiflora* Hangshao' in relation to the time of accumulation of various storage compounds. For this propose, various stages of during seed development were collected for anatomical structure observation, histochemical analysis, and analysis of the accumulation of the main nutrients and fatty acid components. This information will provide a theoretical basis for improving herbaceous peony seed quality and yield in cultivation, and to set a foundation for exploitation and utilization of herbaceous peony seed.

## MATERIAL AND METHODS

### *Plant material*

Seeds of *P. lactiflora* 'Hangshao' were collected from June to August in 2018 at peony germplasm resource garden of Yangzhou University, China (32°39'N, 119°42'E). Seeds were collected at five developmental stages including 30 days after flowering (DAF), 45 DAF, 60 DAF, 75 DAF and 90 DAF (Figure 1). The collected material was placed in ZipLoc plastic bags and taken to the laboratory immediately. Some samples were used immediately for anatomical structure observation, some samples were fixed in formalin-aceto-alcohol for histological staining, and the others were immediately frozen in liquid nitrogen, and then stored at -80 °C until further analysis.

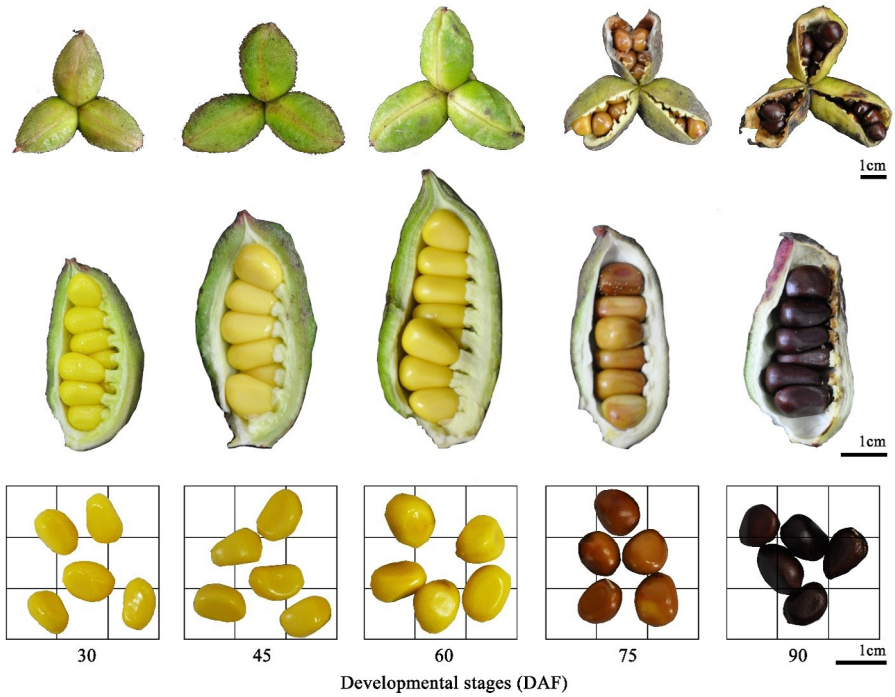


Figure 1. Seeds of collecting period from *Paeonia lactiflora* 'Hangshao'.

### *Anatomical observation*

#### Freehand section

The seeds were cut lengthwise with a sharp blade in the middle, and then placed under a stereoscopic microscope (SZX16, Olympus, Japan) for observation and photography.

#### Observation by $I_2$-KI dyeing

The preparation of $I_2$-KI dye liquor was as following: 2 g KI was completely dissolved in a small amount of distilled water, and then 1 g $I_2$ was added. Next, the mixture was dilute to 300 mL after entirely dissolved by shock. Finally, the dye liquor was stored in brown glass bottle to 4 °C refrigerator for later use.

After cut lengthwise, seeds were put into $I_2$-KI dye liquor for 10 min, then they were removed and placed on a glass slide to observe the morphology under a stereoscopic microscope and take photo.

#### Observation by TTC dyeing

The preparation of TTC dye liquor was as following: 2 g TTC powder was added into a small amount of distilled water, and then was shaken to fully dissolve. Next, the mixture was diluted to 100 mL by distilled water. Finally, the dye liquor was stored in brown glass bottle to 4 °C refrigerator for later use.

After cut lengthwise, seeds were set into TTC incubation solution which has been preheated in a 37 °C water bath. Then, they were gently shaken at intervals of 5 min to make them contact the dye evenly. After 15 min, they were removed and placed on a glass slide to observe the morphology under a stereoscopic microscope and take photo.

### *Histological staining*

Seed sections were cut longitudinally or transversely into 2 mm thick slice from middle and fixed with FAA solution for more than 24 h at 4 °C for later use. Seed slices were dehydrated, made transparent, wax-filled and embedded. Then, the embedded slices were cut into 3 µm sections by slicer (RM2016, Shanghai Laika Instrument Co. LTD, China).

Afterward, the slices were flattened, stuck, dried, dewaxed, rehydrated and finally air dried. To detect starch, the slices were stained with 0.5% (w/v) periodate solution (prepared with 0.3% nitric acid) for 15 min, then washed with distilled water twice. Then, slices were stained with Schiff reagent for 30 min in the dark. Next, the slices were washed with distilled water for 5 min. Lastly, the slices were dried and sealed with glycerine glue. For proteins, the slices were washed with distilled water twice, and were stained by naphthol yellow S for 2 min. Then, the slices were washed quickly with distilled water, and were dehydrated by anhydrous ethanol for two time. Lastly, the slices were dried and sealed with glycerine glue. The slices were examined by light microscape (Eclipse E100, Nikon, Japan) and photographs were taken with a digital camera (DS-U3, Nikon, Japan). To detect lipids, the slices were stained with 10 μM Nile red dye which was dissolved by DMSO. Then the slices were put into 37 °C incubator for 30 min. Next, the slices were dried and were examined by fluorescence microscope (IX83, Olympus, Japan). Lastly, photographs were taken with a digital camera (DS-U3, Nikon, Japan).

### *Determination of weight and water content for seed*

Seeds at different stages of development were taken out from capsules and weighed for fresh weight named $W_1$. Then, they were put in oven at 50 °C for about 72 h and baked to constant weight, which was dry weight named $W_2$. The water content was calculated as followed: Water content = $(W_2-W_1)/W_1 \times 100\%$.

### *Content determination of nutritious substances*

The protein content was determined according to the reagent kit instruction of determination of protein content by commasie brilliant blue staining (Shanghai Cablebridge Biotechnology Co., Ltd.). The determination of starch content was conducted according to the instructions of plant starch content reagent kit (Shanghai Cableridge Biotechnology Co., LTD.). The extraction of crude fat was conducted according to Meng et al. (2021). And the crude fat content was calculated by the following formula $C_f$ (mg/g) = $1000 \times (W_1-W_0) / W_2$. There, $W_0$, $W_1$ and $W_2$ was referred to the weight of receiving flask, the weight of receiving flask and crude fat, and the weight of *Paeonia lactiflora* 'Hangshao' seed powder, respectively.

### *Fatty acid composition analysis and content determination*

Fatty acid composition analysis and content determination mainly includes the following steps such as standard solution preparation, sample hydrolysis, fat extraction, fatty acid esterification, gas chromatographic and mass spectrometric (GC-MS) parameter setting, and calculation for the fatty acid absolute content. The specific methods were referred to previous description (Meng et al., 2021).

### *Statistical analysis*

Experiments described in this study were repeated three times through completely randomized design. Variance analysis was using SAS/STAT statistical analysis software (SAS Institute, Cary, NC, USA). Data shown in figures were means ± SDs (Standard deviation), and the patterns of dry mass, fresh mass, water content, starch content, protein content, lipid content, and fatty acid content were regressed by various functions, such as Linear, Sigmoid, Gaussian and Weibull functions. The equation with the highest $r^2$ was selected.

## RESULTS AND DISCUSSION

### *Anatomical observation of P. lactiflora 'Hangshao'seeds*

Matured seeds of *P. lactiflora* was composed by seed coat, endosperm and embryo. However, the development of these three parts was different. The seed coat was formed in 30 DAF (Figure 2A). However, the endosperm remained free phase in 30 DAF, and the endosperm cellularization was formed in 45 DAF and entered the stage of growth and

differentiation. Nevertheless, the embryo observation was only possible in 60 DAF. With the seed development, the seed coat was thickened first, then hardened by dehydration, and the embryo became bigger after 60 DAF. The results showed that the endosperm development of *P. lactiflora* 'Hangshao' seed was earlier than that of embryo, and the endosperm development was belonged to nuclear endosperm (Sreenivasulu and Wobus, 2013).

In most orthodox (desiccation tolerant) seeds, development can be divided into three stages based on seed size, mass, and storage reserves: early embryogenesis, cell expansion and accumulation of stored reserves and maturation drying. The duration of each of the major phases of development varies from several days to many months, depending on species and prevailing environmental conditions (Bewley et al., 2013). For instance, wheat seeds reached physiological maturity 28 DAF (Nasehzadeh and Ellis, 2017), *Scorpiurus muricatus* 47 DAF and *Lotus ornithopodioides* 54 DAF (Baskin and Baskin, 2014). The time span of seed development in *P. lactiflora* 'Hangshao' from pollination to dispersal was 90 d. Seeds reached physiologically mature by 75 DAF, during which time dry matter content reached its maximum. The early embryogenesis, cell expansion and accumulation of stored reserves and maturation drying of *P. lactiflora* 'Hangshao' seeds occurred at, 0–30, 31–75, and 75–90 DAF, respectively.

In the early stage, there was a lot of starch in the inside cells of the seed coat. With the seed development, the thickness of the seed coat was decreased, and the starch grain was disappeared and were not stained. However, in endosperm, the content of starch grain gradually increased. When the embryo was visible, the blue-black area of the embryo also increased, indicating that the starch in the embryo was accumulating as seed developed (Figure 2B). The red staining of TTC solution on the longitudinal section of *P. lactiflora* 'Hangshao' seed indicated the dehydrogenase activity in the tissue (Figure 2C). During the seed development, seed coat was the least active part of the whole seed and was not stained by TTC, while in the early and middle stage, the dehydrogenase activity of the endosperm was the highest at 45 DAF, and then it began to decline rapidly. In addition, the dehydrogenase activity of the embryo increased

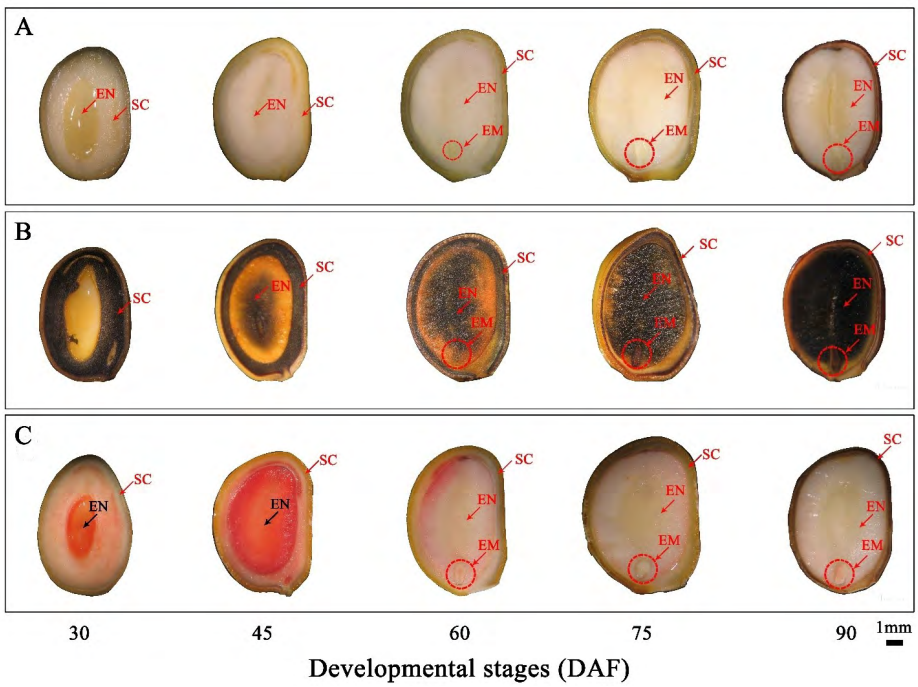


Figure 2. Cross sections of *Paeonia lactiflora* 'Hangshao' seeds in different developmental stages. A: Variation of seeds in different developmental stages; B: $I_2$-KI staining of seeds in different developmental stages; C: TTC staining of seeds in different developmental stages. SC: seed coat; EN: endosperm; EM: embryo.

gradually with the seed development.

### *Histochemical analysis of P. lactiflora 'Hangshao' seeds*

PAS staining (Figure 3A), naphthol yellow S staining (Figure 3B) and Nile red staining (Figure 3C) were used to observe the accumulation of polysaccharides (e.g. starch, sucrose), protein and lipid, respectively. There were a large number of starch grains in the cell of *P. lactiflora* 'Hangshao' seed coat in the early stage, and the starch degraded gradually with the seed development (Figure 3A). The size of starch grains was the largest in 45 DAF. With the seed development, starch grains in endosperm cell began to degrade gradually, and only sporadically distributed to mature stage. After staining with naphthol yellow S, the protein in the tissue presented bright yellow. From Figure 3B, it showed that there was less protein in the seed coat cell at all stages of seed development. However, in the endosperm cell at all stages of seed development, there were more protein which were dyed bright yellow. And with the seed development, the distribution density of protein increased, among which, in 45 DAF, the distribution density was the smallest. In the early stage, the Nile red staining was relatively shallow which indicated the lipid content was less. However, the Nile red staining increased in 60 DAF, and was the deepest in 75 DAF which indicated that the lipid content was the highest in this period. In addition, compared with the 75 DAF, the fluorescence intensity of Nile red staining was slightly reduced in 90 DAF, which indicated that lipid content was reduced (Figure 3C).

### *Weight and water content of P. lactiflora 'Hangshao' seeds*

The fresh ad dry weight of 100-seeds of *P. lactiflora* 'Hangshao' seed first increased and then decreased slightly in the seed development, and the maximum value appeared in 75 DAF, which was 47.57 g and 26.34 g, separately. At 90 DAF, the fresh weight and dry weight of 100 mature seeds were 41.67 g and 25.92 g separately. However, the water content of *P. lactiflora* 'Hangshao seeds had been decreasing all the time and reached 37.8% at 90 DAF of seed maturity (Figure 4). In the first stage of development, seed fresh mass of *P. lactiflora* 'Hangshao' increased but the

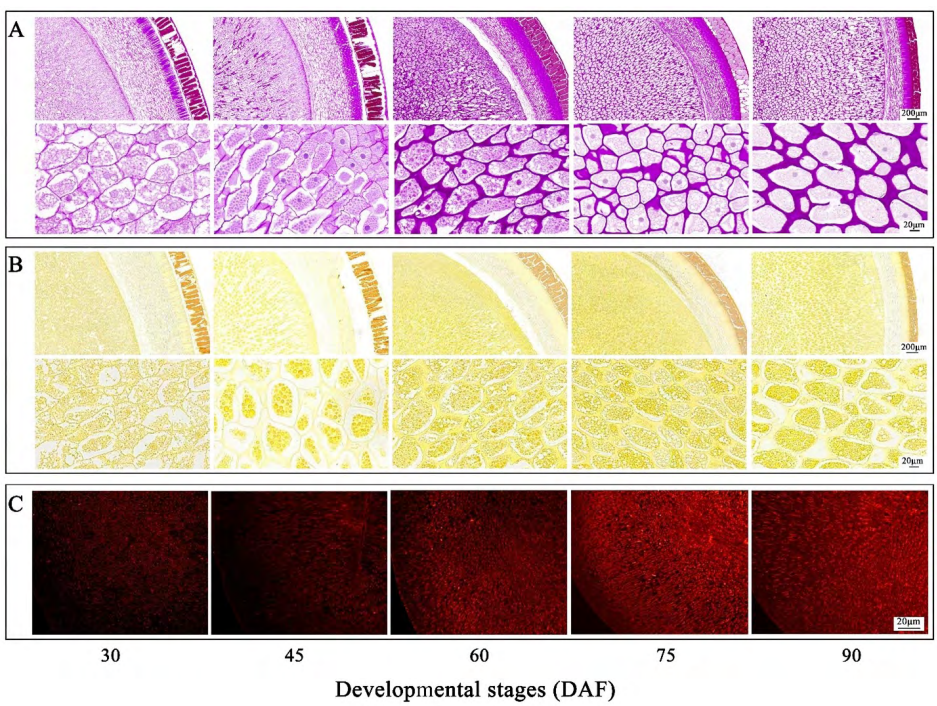


Figure 3. Histochmeical tests and anatomic features of cross sections of *Paeonia lactiflora* 'Hangshao' seeds in different developmental stages. A: polysaccharides (e.g. starch, sucrose) observation by PAS staining; B: protein observation by naphthol yellow S staining; C: lipid observation by naphthol yellow S staining.

speed is slow; the main storage reserves was soluble sugars. At the second development stage, seed dry weight quickly increased due to the synthesis and deposition of stored reserves; the seed water content of *P. lactiflora* 'Hangshao' also decreased, possibly due to the displacement of water content of insoluble reserves from cytoplasm (Bewley et al., 2013). During the third stage, dry mass of *P. lactiflora* 'Hangshao' remained constant while fresh mass decreased, and this often explained as the seeds underwent an acute loss of water because of a loss of vascular supply to the seed. Another changed during maturation is seed color changed from yellow to dark; this is probably caused by polyphenolic compound oxidation at palisade layer (Werker, 1997), as seed coat of *P. lactiflora* 'Hangshao' is abundant in polyphenolic compounds (unpublished data).

Generally speaking, the water content of the orthodox seeds after maturation is less than 10% (Roberts, 1973). Although the water content of mature *P. lactiflora* 'Hangshao' seeds is very high, close to 40%, that does not mean that *P. lactiflora* 'Hangshao' seeds belong to the recalcitrant seed. The reasons are that (1) the development of recalcitrant

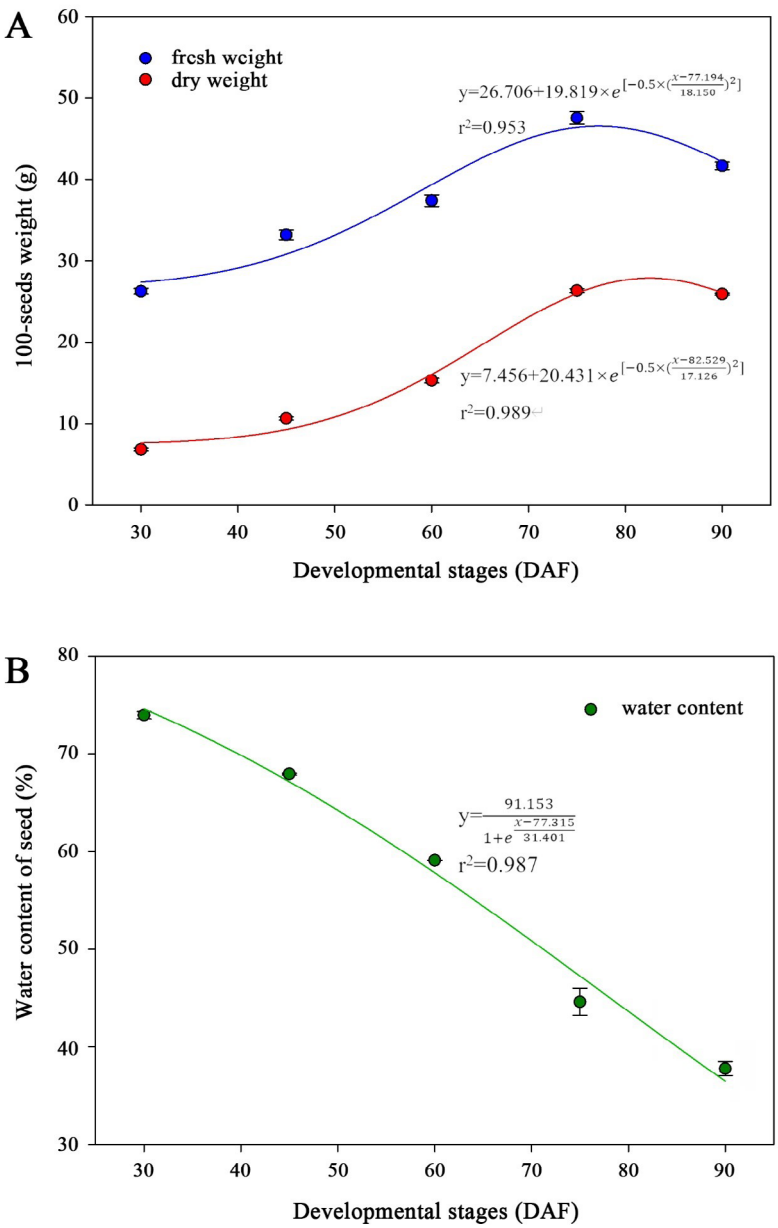


Figure 4. 100-seeds weight and water content *Paeonia lactiflora* 'Hangshao' seeds in different developmental stages. A: 100-seeds weight; B: water content of seed.

seeds does not go through the stage of maturation drying (Pammenter and Berjak, 1999). At the later stage of development, the dry weight of *P. lactiflora* 'Hangshao' seeds is stable, but the fresh weight of that decreases, indicating that the water content decreases sharply, which is different from that of the recalcitrant seeds. (2) Recalcitrant seeds can't tolerate dehydration (Berjak and Pammenter, 2013), and will die if they are dehydrated such as *Litchi chinensis* (Zhang et al., 2015), so it is difficult to store them. However, *P. lactiflora* 'Hangshao' seeds can be stored normally, and they can germinate in the second year after harvesting. Therefore, *P. lactiflora* 'Hangshao' seeds don't belong to the recalcitrant seed. As to whether it belongs to the orthodox seed or the intermediary seed, further desiccation experiments is needed.

### *Dynamic change of nutrient content in P. lactiflora 'Hangshao' seeds*

The content of starch in *P. lactiflora* 'Hangshao' seeds showed an increased and then decreased pattern. The maximum value was in 60 DAF (Figure 5A). The content of protein in *P. lactiflora* 'Hangshao' seed first decreased, and then increased (Figure 5B). The content of lipid in *P. lactiflora* 'Hangshao' seed first increased, and then decreased slightly (Figure 5C). The maximum value occurred in75 DAF.

During development of *P. lactiflora* 'Hangshao' seeds, starch content increased gradually until 60 DAF, but they decreased when accumulation of storage oil and protein increased. Such a pattern has already been observed in the *Arabidopsis thaliana* (Baud et al., 2002), *Brassica napus* (Silva et al., 1997) and *Sinapis alba* seed (Fisher and Schopfer, 1988). However, in cereal seeds, such as those of *Oryza sativa* and *Triticum aestivum*, in which starch is the main storage reserve starch, starch content does not decrease (Bewley et al., 2013). During the development of *P. lactiflora* 'Hangshao' seeds, starch accumulates first. Because soluble sugar is a major product of photosynthesis as a metabolic substrate for seed respiration and metabolism (Haughn and Chaudhury, 2005). The excessive soluble sugars were transferred and stored as starch once seed growth needs were satisfied. Starch would be decomposed afterwards, as they functioned as precursors in oil and protein synthesis. Previous study has shown that oligosaccharides, such as raffinose and stachyose, constitute the critical component in acquiring desiccation tolerance (Baud et al., 2002). Seeds of *P. lactiflora* 'Hangshao' are orthodox, but little is known about the soluble sugar composition during *P. lactiflora* 'Hangshao' seeds.

During the seed development of *P. lactiflora* 'Hangshao', the protein content slightly decreased and then gradually increased. Because the main components of soluble protein include various types of enzymes, the result show that biochemical reactions should increase significantly during seed development (Bewley et al., 2013). Furthermore, reserve proteins store nitrogen and sulfur, which are essential for seed germination and seedling growth (Bewley et al., 2013). The crude fat content in seeds of *P. lactiflora* 'Hangshao' continually increased, but the content revealed a trend of slight decline in late maturity stage. A similar oil accumulation pattern is found from many oilseeds like *Arabidopsis thaliana* (Baud et al., 2002), *Brassica napus* (Silva et al., 1997), *Glycine max* (Yazdi-Samadi et al., 1977) and *Sinapis alba* seed (Fisher and Schopfer, 1988). This decline possibly is due to the maturing seeds losing trophic supply from plants and need to consume a proportion of lipid reserves, when synthesizing protein and carrying out metabolic reactions (Baud et al., 2002). In oilseeds, lipids are the major carbon reserve for germinating and growing seedlings (Bewley et al., 2013). Furthermore, from an ecological perspective, the fat might act as protection against the low-temperature during seeds dispersion, in the autumn or winter seasons, but for this confirmation ecological studies should be performed.

### *Fatty acid components and content of P. lactiflora 'Hangshao' seed*

There were 26 types of fatty acids were detected and characterized, including octanoic acid (C8:0), decanoic acid (C10:0), lauric acid (C12:0), ficocerylic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0, PA), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), cis-10-heptadecenoic acid (C17:1), stearic acid (C18:0, SA), oleic acid (C18:1N9C, OA), trans-oleic acid (C18:1N9T, TOA), linoleic acid (C18:2N6C, LA), trans-linoleic acid (C18:2N6T), α-linolenic acid (C18:3N3, ALA), γ-linolenic acid (C18:3N6), arachidic acid (C20:0), eicosenoic acid (C20:1),

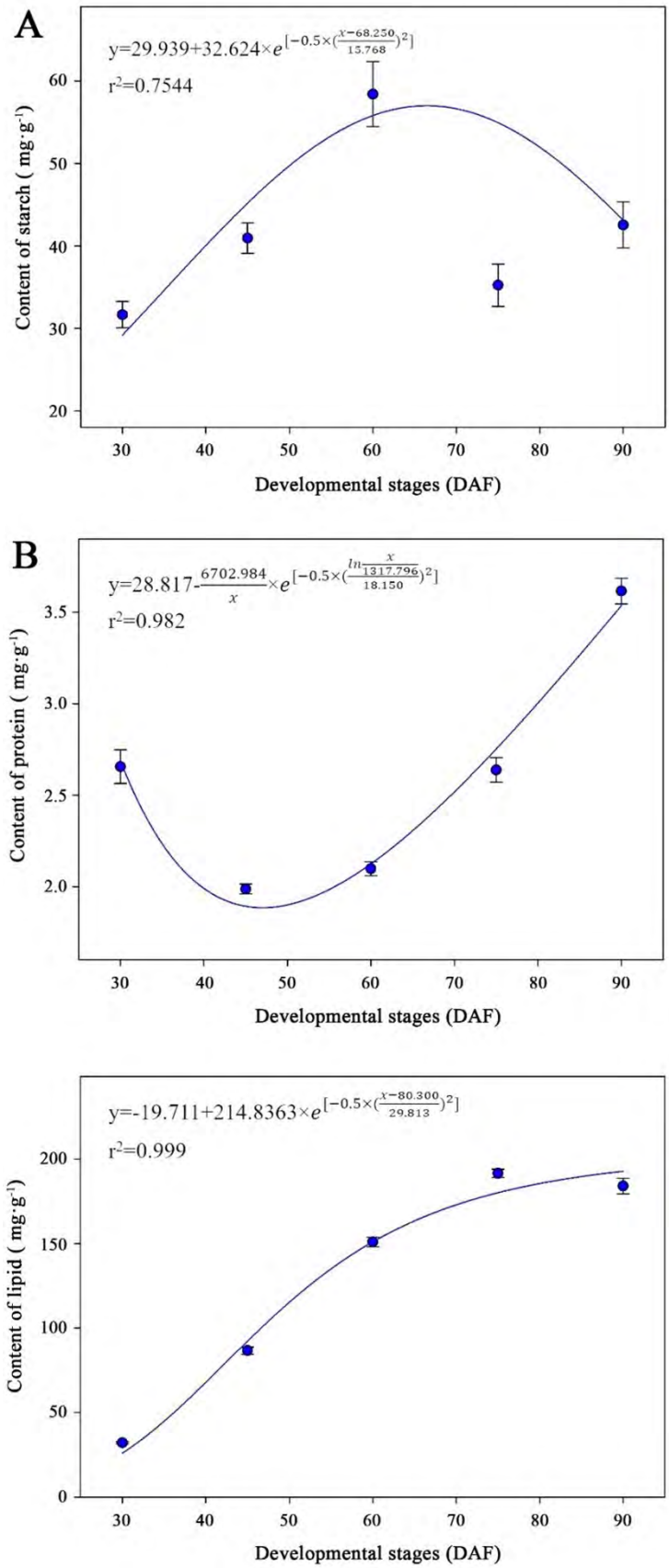


Figure 5. Content variation of storage compounds of *Paeonia lactiflora* 'Hangshao' seeds in different developmental stages. A: content of starch; B: content of protein; C: content of lipid.

eicosadienoic acid (C20:2), cis-11,14,17-eicosatrienoic acid (C20:3N3), heneicosanoic acid (C21:0), docosanoic acid (C22:0), erucic acid (C22:1N9, EA), tricosanic acid (C23:0) and tetracosanoic acid (C24:0).

There were 7 types of fatty acids which content was more than 100 mg.Kg$^{-1}$ in each stage, and they were PA, SA, OA, TOA, LA, ALA and EA (Table 1, Figure S1). The rank of the content of 7 types of fatty acids in 5 stages was all ALA>LA>OA>PA>SA>TOA>EA. Variation of fatty acid (FA) content in the *P. lactiflora* 'Hangshao' seed was shown in Figure 6. During the development, saturated fatty acid (SFA) content was relatively low, showing an increasing trend (Figure 6A). However, the total FA and unsaturated fatty acid (UFA) content were increased first and then decreased, reached the maximum values in 75 DAF, which were all more than 250,000 mg.Kg$^{-1}$. The content of

Table 1. Content of 26 fatty acids in *Paeonia lactiflora* 'Hangshao' seedestimated by gas chromatography and mass spectrometric (GC-MS)*.

| Stage<br>Content (mg.Kg$^{-1}$) fatty acid | 30 DAF | 45 DAF | 60 DAF | 75 DAF | 90 DAF |
|---|---|---|---|---|---|
| octanoic acid (C8:0) | 1.77± 0.00 | 0.73 ± 0.00 | 1.08 ± 0.00 | 1.59 ± 0.40 | 0.98 ± 0.00 |
| decanoic acid (C10:0) | 1.34 ± 0.00 | 0.74 ± 0.00 | 1.10 ± 0.00 | 1.21 ± 0.00 | 0.99 ± 0.00 |
| lauric acid (C12:0) | 4.99 ± 0.00 | 4.75 ± 0.21 | 7.41 ± 0.16 | 8.17 ± 0.00 | 9.18 ± 0.19 |
| ficocerylic acid (C13:0) | 1.36 ± 0.00 | - | 0.84 ± 0.00 | 0.82 ± 0.00 | 1.01 ± 0.00 |
| myristic acid (C14:0) | 28.22 ± 0.27 | 39.14 ± 0.44 | 75.15 ± 0.86 | 73.96 ± 0.63 | 72.09 ± 0.34 |
| pentadecanoic acid (C15:0) | 18.48 ± 0.26 | 22.31 ± 0.38 | 22.53 ± 0.32 | 18.82 ± 0.63 | 22.60 ± 0.20 |
| palmitic acid (C16:0) | 2480.85 ± 1.66 | 4998.41 ± 48.85 | 7145.15 ± 32.94 | 7433.89 ± 34.90 | 7187.24 ± 63.24 |
| palmitoleic acid (C16:1) | 38.31 ± 0.53 | 28.97 ± 0.96 | 43.53 ± 0.81 | 43.30 ± 0.63 | 70.29 ± 0.71 |
| heptadecanoic acid (C17:0) | 17.38 ± 0.27 | 71.18 ± 0.66 | 110.40 ± 2.26 | 106.10 ± 1.46 | 133.52 ± 1.54 |
| cis-10-heptadecenoic acid (C17:1) | 16.27 ± 0.27 | 73.61 ± 0.79 | 159.49 ± 1.14 | 155.95 ± 1.45 | 171.49 ± 0.71 |
| stearic acid (C18:0) | 247.21 ± 1.42 | 904.73 ± 4.96 | 1418.09 ± 13.81 | 1654.98 ± 0.96 | 2442.28 ± 8.72 |
| oleic acid (C18:1N9C) | 7603.58 ± 17.13 | 25457.75 ± 69.35 | 44653.55 ± 83.79 | 57114.56 ± 57.10 | 60776.93 ± 52.30 |
| trans-oleic acid (C18:1N9T) | 239.40 ± 5.33 | 667.18 ± 5.38 | 1125.17 ± 3.02 | 1227.78 ± 12.12 | 1276.94 ± 5.89 |
| linoleic acid (C18:2N6C) | 13784.64 ± 41.62 | 31912.13 ± 22.68 | 65584.79 ± 345.56 | 80707.16 ± 103.83 | 71857.15 ± 71.50 |
| trans-linoleic acid (C18:2N6T) | 26.16 ± 0.53 | 80.70 ± 1.60 | 186.97 ± 6.03 | 220.54 ±.00 | 192.24 ± 4.44 |
| α-linolenic acid (C18:3N3) | 20824.01± 143.65 | 58429.80 ± 367.63 | 93140.17 ± 226.03 | 114224.92 ± 200.74 | 105274.82 ± 1843.10 |
| γ-linolenic aicd (C18:3N6) | 3.68 ± 0.00 | 384.84 ± 6.53 | 88.33 ± 1.45 | 38.49 ± 1.80 | 534.60 ± 18.72 |
| arachidic acid (C20:0) | 29.13 ± 0.53 | 124.02 ± 1.17 | 118.45 ± 1.46 | 115.00 ± 1.11 | 227.37 ± 0.87 |
| eicosenoic acid (C20:1) | 40.23± 0.27 | 238.80 ± 1.89 | 505.90 ± 5.99 | 609.64 ± 3.35 | 734.85 ± 6.21 |
| eicosadienoic acid (C20:2) | 12.35 ± 0.54 | 27.39 ± 0.22 | 60.77 ± 1.18 | 67.09 ± 0.84 | 73.31 ± 1.23 |
| cis-11,14,17-eicosatrienoic acid (C20:3N3) | 6.63 ± 0.27 | 17.07 ± 0.96 | 17.67 ± 0.65 | 17.91 ± 1.11 | 18.84 ± 0.59 |
| heneicosanoic acid (C21:0) | 1.39 ± 0.00 | 5.49 ± 0.23 | 5.12 ± 0.29 | 6.27 ± 0.42 | 9.39 ± 0.20 |
| docosanoic acid (C22:0) | 18.50 ± 0.27 | 38.49 ± 1.39 | 37.69 ± 0.75 | 39.73 ± 0.87 | 97.99 ± 2.15 |
| erucic acid (C22:1N9) | 112.31 ± 2.56 | 159.91 ± 0.97 | 107.12 ± 2.72 | 181.03 ± 1.69 | 220.79 ± 1.95 |
| tricosanic acid (C23:0) | 9.62 ± 0.54 | 6.91 ± 0.39 | 26.21 ± 0.75 | 17.17 ± 0.42 | 57.60 ± 0.71 |
| tetracosanoic acid (C24:0) | 31.32 ± 0.49 | 106.59 ±4.83 | 96.67 ± 5.15 | 114.90 ± 2.30 | 227.56 ± 5.66 |

*Values were the mean ± SD (n=3);- represented the content < 0.5 mg.Kg$^{-1}$.

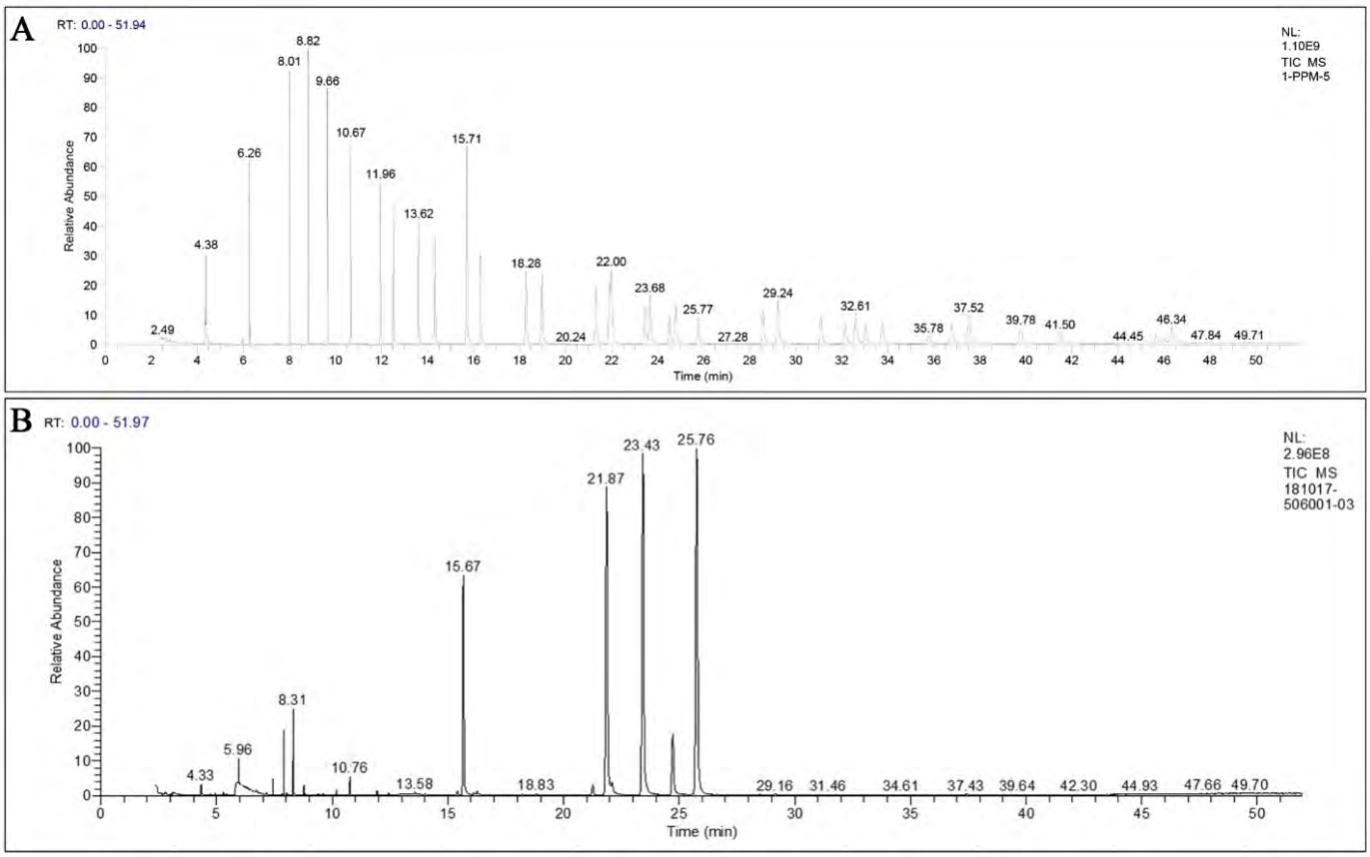


Figure S1. Gas chromatogram profile of 35 fatty acid for mixed standard and fatty acid for *Paeonia lactiflora* 'Hangshao' seed A: 35 fatty acids for mixed standards; B: fatty acids extracted from *Paeonia lactiflora* 'Hangshao' seed.

polyunsaturated fatty acid (PUFA) was about 3 times higher than that of monounsaturated fatty acid (MUFA) during the seed development (Figure 6B).

In the mature stage, the content of ALA, LA and OA exceeded 60000 mg.Kg$^{-1}$ in the *P. lactiflora* 'Hangshao' seed. OA, LA and ALA were belonged to UFA, which played an important role in human nutrition and physiological performance (Hagve, 1988). OA could regulate the level of high- and low-density lipoprotein in human body, maintain the balance of lipoprotein content, thereby slowing down atherosclerosis and effectively preventing the occurrence of cardiovascular disease (Parthasarathy et al., 1990; Jones et al., 2014). LA and ALA were essential fatty acid for human, which cannot be synthesized in the human body and must be obtained through diet, such as plant oil, deep-sea fish oil and so on (Spector and Kim, 2015). LA was the precursor substance of ω-6 PUFAs, and was associated with obesity, coronary heart disease, diabetic nephropathy, cancer, blood pressure and other physiological function (Zock and Katan, 1998; Harris et al., 2007; Santos et al., 2018; Naughton et al., 2018; Nunes et al., 2018).

ALA was the precursor substance of ω-3 PUFAs and can synthesize Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (Spector and Kim, 2015). ALA was an important substance of cell membrane and biological enzyme (Bjerve et al., 1987), which has a wide range of physiological functions in preventing cardiovascular disease, cancer, inflammation, and allergy, providing neuroprotection, enhancing immunity, and protecting retina and brain development (Barceló-Coblijn and Murphy, 2009). Although both LA and ALA were essential fatty acids for human body, they used the same desaturatase and elongation enzyme in the metabolic transformation process of human body, so there existed metabolic competition inhibition between the two fatty acids (Wang, 2015). Therefore, the ω-6/ω-3 ratio in the diet had different effects on physiological functions such as lipid metabolism, immune function, and antioxidant (Su and Guo, 2003). Studies had shown that when the ω-6/ω-3 PUFAs intake ratio was 1:1, it played the most significant role

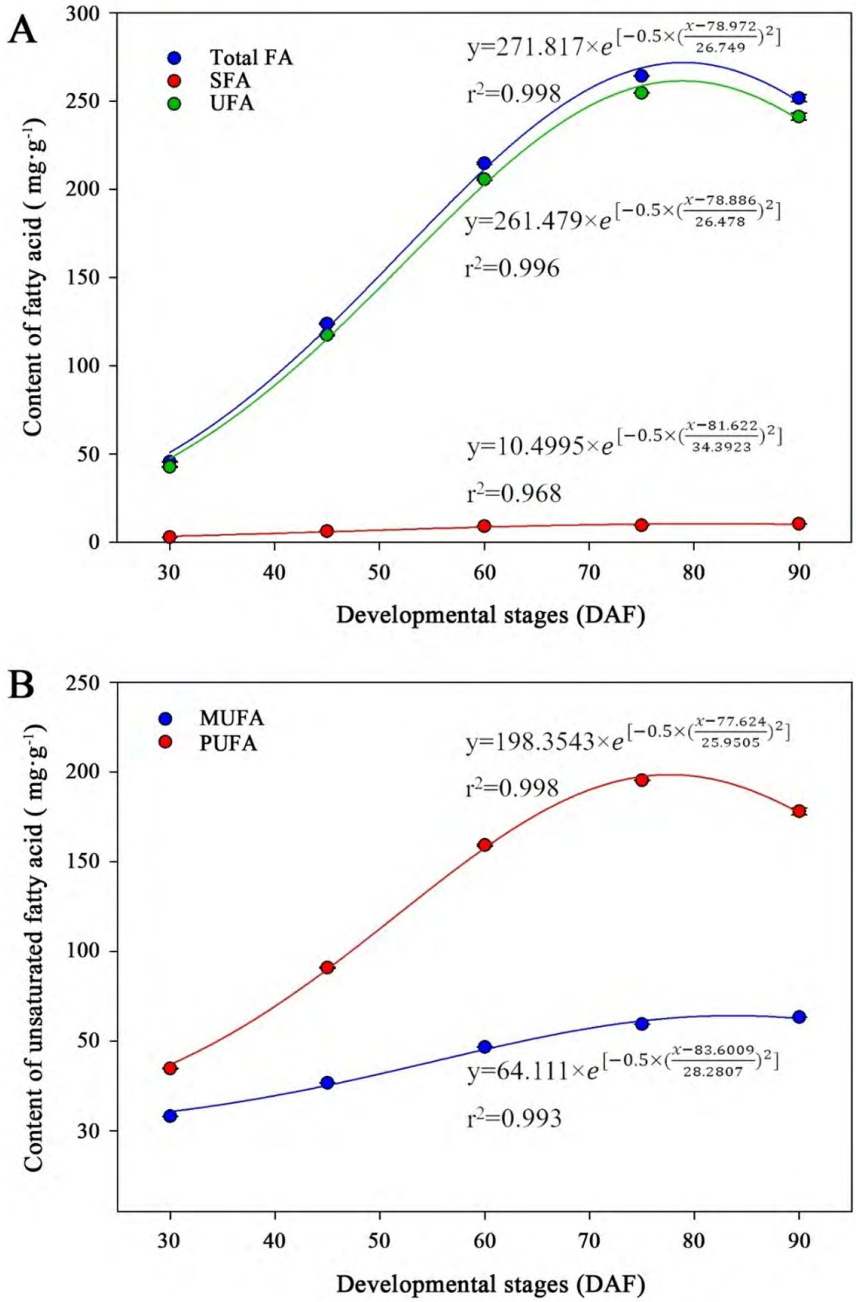


Figure 6. Content of fatty acid of *Paeonia lactiflora* 'Hangshao' seeds in different developmental stages. A: Total FA, SFA and UFA content; B: MUFA and PUFA content. FA: fatty acid; SFA: saturated fatty acid; UFA: unsaturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

in physiological function (Guo and Su, 2004; Yang, 2017; Jin et al., 2019). In this study, the content of LA and ALA in mature seed of *P. lactiflora* 'Hangshao' was 71857.15 mg.Kg$^{-1}$ and 105274.82 mg.Kg$^{-1}$, respectively, which ratio was about 0.7:1 and was close to 1:1. The accumulation and transformation of nutrients in oil plants were the theoretical and practical basis for the formation of yield and quality. It has been proposed that starch synthesis competes with lipid synthesis and therefor restricts lipid synthesis in seeds (Bettey and Smith, 1990). In oil seeds such as rape and *Arabidopsis*, starch accumulates a lot in the early stages of development while lipid do not change significantly, and when starch content accumulates to the maximum while lipid enters into the period of rapid accumulation, however,

when lipid content accumulates to the maximum while starch is absent from mature seeds (Silva et al., 1997; Focks and Benning, 1998; Vigeolas et al., 2004).

Previous studies have showed that early accumulation and later degradation of starch also provide many precursors for lipid synthesis (Periappuram et al., 2000; Hill et al., 2003). However, previous research on herbaceous peony seed mainly focused on dormancy and germination (Zhang et al., 2019), and only a few researches focused on its oil component (Ning et al., 2015; Meng et al., 2017), little is known about the accumulation of storage reserves, especially for its fat acid composition. In this study, we found out the accumulation characteristics of three storage compounds such as starch, protein and lipid in herbaceous peony 'Hangshao' seed with the seed development. Our study filled this gap and will provide a theoretical basis for improving herbaceous peony seed oil quality and yield in cultivation such as the selection of fertilization type and time, the selection of harvest time.

## CONCLUSIONS

Mature seed of *P. lactiflora* 'Hangshao' was composed of seed coat, embryo and endosperm, and the endosperm belonged to the karyotype endosperm. The time span of seed development for *P. lactiflora* 'Hangshao' was 90 DAF, with seeds becoming physiologically matured at 75 DAF. The whole development progress can be divided into early embryogenesis, cell expansion and accumulation of stored reserves and maturation drying. Lipid was the main storage reserve of mature seeds. A total of 26 kinds of fatty acid were detected by gas chromatography and mass spectrometer, and among which, seven kinds was all more than 100 $mg.Kg^{-1}$ in all developmental seeds.

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