



Full Length Article

Mineral Composition and Accumulation of *Paeonia ostii* Seeds Oil: Contribution to Daily Minerals Intake

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Abstract

Paeonia ostii seed oil contains a large amount of unsaturated fatty acid and few studies have been focused on it. In this study, accumulation patterns of mineral elements was showed through dissecting and observing *P. ostii* seed, extracting *P. ostii* seed oil and analyzing its mineral elements and expression of genes related to mineral elements transportation. The results showed that number and size of oil bodies in *P. ostii* seed keeps growing during seed maturation process, concentration of mineral elements in *P. ostii* seed oil keeps accumulating during development process and reaches stable state during the seed maturing stage. Tendency of 14 transport genes related to mineral elements varies during seed maturation process, thus, providing theoretical basis for further research of *P. ostii* seed oil mineral elements. © 2018 Friends Science Publishers

Keywords: *Paeonia ostii*; Edible oil; Mineral

Introduction

There are three main sources of edible vegetable oil: herbal, woody and fuel crops. Compared with soybean, rape and peanut edible oil plants, woody edible oil plants have the advantages of strong ecological function: no occupation of cultivated land, easy management, and excellent oil quality, high nutrition, less investment, large benefit and long profit period. Tree peony (*Paeonia section Moutan* DC.) is Chinese original woody precious flower. After thousands of years of natural growth and 1500 years of artificial cultivation history, tree peony is acclimated as a famous ornamental flower around world. Recently, according to its high α -linoleic acid content, tree peony seeds oil is developed as a new source of woody edible oil. The legume of tree peony has a star shaped fruit which are rich of unsaturated fatty acids. Compared to common plant oil, for example, soybean oil, peanut oil, rapeseed oil, castor oil, sunflower oil and corn oil, seed oil from tree peony (*Paeonia ostii*) have been authenticated as original food resources containing α -linolenic acid (ALA), according to the Ministry of Health in China recently (Li *et al.*, 2015; Su *et al.*, 2016). Besides unsaturated fatty acids, it is generally known that mineral composition in plant oils are also essential dietary nutrients (Zouari *et al.*, 2016). However, at present, many studies are focusing on organic components of edible vegetable oil, few research paid attention to the metal components (Santana *et al.*, 2016). To meet the people's nutritional requirements, studies also need to

investigate and explore new oil sources which can meet the mineral balance need of human body. However, there are few studies about mineral elements in plant oil (Biedermann and Grob, 2015; Singh *et al.*, 2016).

It is well known that normal growth of all plants required macro- and microelements. As sessile organisms, plants are also frequently protecting cells from toxicity resulting from metal excess. Within the cells and tissues, mineral elements required for regulating system of nutrient absorption and distribution. Mineral elements are an important component of cell survival, especially during the process of protein-mineral interactions (Andersen *et al.*, 2016). For example, during the interaction between enzymes and its substrate, Ca^{2+} , Cu^{2+} , Mg^{2+} , K^{+} and Na^{+} can be a cofactor in enzymatic reactions. These mineral elements can also maintain the pH and osmotic pressure and normal physiological activity within the cell.

Calcium is a common mineral content in plant oil (Vandana and Bhatla, 2009). Calcium signals are core regulators and transducers in many processes of plants. Ca^{2+} can easily form compounds with membranes, proteins, and organic acids (Kudla *et al.*, 2010). The Ca^{2+} /Cation Antiporter (CaCA) superfamily was discovered in almost all kingdoms of life as an old and wide family of ion-coupled cation transport proteins (Cai and Lytton, 2004). The CAX (for Cation Exchanger) family is one of the five families that constitute the CaCA superfamily (Shigaki *et al.*, 2006). Expression of *Arabidopsis* CAX2 in transgenic tobacco enhanced manganese tolerance and changed metal

accumulation (Hirschi *et al.*, 2000). Veatch-Blohm *et al.* (2013) reported that the calcium to magnesium ratio affected a cross-tolerance to nutrition deficiency in *Arabidopsis lyrata* ssp. *Lyrata* (Veatch-Blohm *et al.*, 2013). Plant growth and development require iron and have evolved various strategies to cope with Fe homeostasis *in vivo* (Wang *et al.*, 2015). It was reported that iron nutrition influences the accumulation and toxicity of cadmium in rice plants (Isayenkov *et al.*, 2010). Copper (Cu) performs array of functions in plants containing photosynthesis, flowering, cell wall remodeling and seed set (Jung *et al.*, 2014). Interestingly, *P. ostii* can grow normally on Cu mining areas with lower Cu contents than bare land (Wang *et al.*, 2016). However, the mechanism of the survival strategy on Cu mining is still not clear. Na⁺ transport between cells including several pathway can perform from cells at the plasma membrane via Na⁺/H⁺ antiport (Shi *et al.*, 2000), also can be transported through sodium-activated potassium channels (Rizzi *et al.*, 2016), e.g., Na⁺-K⁺-ATPase, a new kind of plasma membrane receptor (Aperia *et al.*, 2016). Manganese (Mn) is also an important metal for plant growth, a number of manganese transporters involved in the translocation to the shoot, have been identified in *Arabidopsis* and rice (Ueno *et al.*, 2015). Some transporter families were discovered in Mn transport in plants and are linked to each other, Such as natural resistance-associated macrophage protein (NRAMP), IRT-like protein (ZIP), ZRT and so on. Although, the characteristics of Mn transporters in certain plants, still have limited knowledge about the roles of Mn transporters in numerous plant species (Ueno *et al.*, 2015). In our study, we also focused on mineral composition, accumulation and transportation at different development stages of *P. ostii* seeds. We aimed to (1) provide information of composition and accumulation on mineral elements in *P. ostii* seeds oil; (2) screen out a series candidate genes related to mineral transportation.

Materials and Methods

Plant Materials

Paeonia section Moutan DC. was grown in the germplasm repository of horticulture and plant protection college, Yangzhou University, Jiangsu Province, P. R. China (32°23'31"N, 119°24'50"E). It was introduced to the plantation and grown in the same ecotype and planting conditions for 3 years. The peony seed development process was surveyed from fertilization till maturation from May to August, 2015. Pods were hand-collected at 70, 85 and 100 days after pollination (DAP, immature stage). Pods with the same developing stage from three plants were composed of three replicates. Moisture content was estimated by the loss of weight over drying in an oven at 100°C to a standing weight. Seed samples were quick frozen in liquid nitrogen and then stored at -80°C freezer until further RNA extraction using.

Anatomical Observations

For microscopic observation, tissue samples were fixed in the 2.5% glutaraldehyde about 2 h and stored at 4°C. Washed with 0.1 M PBS 15 min, then fixed in 1% osmium tetroxide for 3 h, wash with 0.1 M PBS for 15 min. For observation by transmission electron microscope (TEM), the fixed seeds were dehydrated in 50%, 70%, 80%, 90%, 95%, and 100% ethanol for 15 min. Dehydration in 100% acetone for 15 min at RT, following dehydration in 100% acetone adding anhydrous sodium sulfate for 15 min. After infiltration, samples were embedding in board and polymerized under oven, for 12 h at 37°C, then for 48 h at 60°C. Then sliced and observed by TEM (Tecnai 12, Philips, Netherlands).

Extraction of Seed Oil

The dried seed samples of different stage were peeled and pressed into powdered form. The oil content of developing seeds was extracted as described in our former study (Ning *et al.*, 2015). Oil was extracted through ultrasonic assisted extraction method. 30.0 g of seed powder was weighed and then transferred into a 500 mL round bottom flask. The proportion of n-hexane and material was 1:13 mixed together (g/mL). Afterwards, the flask was linked to a condenser tube and put in the ultrasonic cleaning machine to extract oil at 70°C and 200 W of power at a frequency of 80 kHz. After extracting for 1.5 h, the mixture was centrifuged at 4000 rpm for 10 min. Then the supernatant was separated and dried through rotary evaporation at 50°C for n-hexane recovery and oil separation. The calculation method of oil yield was as follows:

$$\text{Oil yield (\%)} = \frac{\text{Seed oil weight (g)}}{\text{Seed weight (g)}} \times 100$$

Mineral Analysis

We used Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Perkin-Elmer, Elan DRC-e) and by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Perkin-Elmer, Optima 7300 DV) to measure the mineral concentration in the seed oil. The mineral concentration in the seed oil was measured by using criterions used in the analysis from atomic spectrometry standards of Perkin-Elmer, SPEX, Accu Standard and Merck.

Accurately 0.50 g oil sample was weighed under test in the 50 mL porcelain crucible. After continuously heated by electric stove, sample was carbonized as black sheet. Then the sample was sealed by lid of porcelain crucible, and burned in muffle furnace at 600°C for 10 h until oil disappear entirely. The crucible wall and cover were rinsed by 2–3 mL of pure nitrate, then washed with 10 mL ultrapure water and transferred to flask diluted to 50 mL volume, and mixed. The liquid mixture was filtered by 0.45 μm membrane filter of water-phase, and measured through

ICP-MS. Atomizer flow was 0.92 L/min; auxiliary gas flow rate of 1.2 L/min, the plasma gas was 16 L/min, radio-frequency power of generator was 1100 w. Other mineral elements composition was detected through ICP-OES.

Expression Analysis of Mineral Transport Related Genes

RNA sample of each time point was collected from three independent plants and extracted by using a plant RNA Extraction Kit (TaKaRa) following the manufacturer's protocol. The cDNA was synthesized from 1 µg RNA by PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Japan) and quantified with a spectrophotometer (Eppendorf, Germany) at 260 nm. Real-time quantitative RT-PCR analyses were conducted according to the MIQE guidelines. We selected 14 mineral transportation related genes from transcriptome of *P. ostii* seeds (<http://www.biomedcentral.com/content/supplementary/s12864-015-1429-0-s2.zip>). All primers were designed through Vector NTI version 11.0 software (<http://www.lifetechnologies.com/cn/>) and commercially synthesized (Genery, China) (sequences presented in Table 1). Quantitative RT-PCR analysis was carried out by using the SYBR Premix Ex Taq™ Kit (TaKaRa), according to the manufacturer's protocol, with three technical replicates by using a BIO-RAD CFX96™ Real-Time System (C1000™ Thermal Cycler) (Bio-Rad, USA). The amplification was performed under the following conditions: 50°C for 2 min, followed by an initial denaturation step at 95°C for 5 min, 40 cycles at 95°C for 15 s, 72°C for 40 s and 55°C for 15 s. Following amplification, all products came under melt curve analysis. A negative control lacking a cDNA template was performed to assess the overall specificity. *P. ostii* ubiquitin gene was used for an internal reference gene (Table 1).

Results

Oil Accumulation at Different Stages of Seed Development

Seeds of *P. ostii* were immature at 70 days and attained at maturity in 85 days. At 100 days, the pods were cracking and seeds were fully matured (Fig. 1a). From 70 days to 100 days, the water content gradually decreased, however, the amount of oil has been reaching stable (Fig. 1b). Seeds at 70 days content 55.44% moisture, until to 100 days the water content decreased rapidly to about 28.41%. oil content at 70d was 31.39%, from 70 to 100 days remained around 30%.

The TEM observation showed that in endosperm cells of *P. ostii* seeds, the number and size of oil body showed increasing trend from 70d to 100d (Fig. 2). At the same time, it has been observed that the vacuoles size in 85 days, get wider and by the 100 day volume of vacuoles decreased.

Mineral Composition of *P. ostii* Seeds Oil

The content of mineral elements increased at seed maturation stage. Ca, K and Na content were higher than the other mineral element in the seed oil. The content of Ca in the seed oil in 70 d was 59.55 µg/g, it rapidly increased to 240.2 µg/g at 85 d, and reached to 254.45 µg/g in 100 d. The content of K at 70 d was 30 µg/g, and it rapidly increased to 125 µg/g at 100 d. The Mg content was only 10 µg/g at 70 d, however, it increased 10 times after 30 days, it reached to 100 µg/g at 100 d. From 70 d to 100 d, the content of Fe changed inconspicuously; it increased from 8 µg/g to the final 12 µg/g. At 70 d, the Zn was only 3 µg/g, it increased by 3 times at 100 d, and finally it reached 9 µg/g. The change of Cu was also not obviously; it was 6 µg/g at 70 d and increased to 7 µg/g at 100 d. The content of Mn in 70 d was around 0.1 µg/g, and rapidly increased to 3.5 µg/g at 100 d. Ni content was the lowest in the numerous elements, only 0.18 µg/g at 70 d, and this number doubled to 0.36 µg/g at 100 d (Fig. 3).

Mineral Transport Related Genes

To explore the relationship between mineral elements accumulation and its transport-related genes, we selected 14 mineral transport related genes from National Center for Biotechnology Information (NCBI) Short Read Archive (accession number: SRP051810).

These 14 tested genes include three Ca transport-related genes, three Fe transport-related genes, three K transport-related genes, four Mg transport-related genes and one Zn transport-related gene (Table 2). The results of Quantitative Real-time PCR showed that, relative expression of *Cal* (CAX) is gradually decreasing, the expression peak of *Ca2* (Sodium/calcium exchanger family protein) occurred in 85 days, *Ca3* (C2 calcium/lipid-binding plant phosphoribosyltransferase) expressed highest in 100 days. Among iron transport-related genes, *Fe1* ((2OG) and Fe (II)-dependent oxygenase) expression showed a downward trend, while relative expression of *Fe2* (Oxidoreductase, 2OG-Fe (II) oxygenase_family) and *Fe3* (FC2 FC-II, ATFC-II ferrocyclase 2) gradually raised. During 70 days to 85 days *K1* (AKT5 KT5 K⁺ transporter 5) had a weak rise and rapid decline in 100 days. While *K2* (ATKEA4 KEA4 K⁺ efflux antiporter) and *K3* (KUP11 K⁺ uptake permease) showed a declining trend. *Mg1* (Magnesium transporter CorA-like_family), *Mg2* (MGT2 magnesium transporter 2) and *Mg3* (Oligosaccharyltransferase complex/magnesium transporter family) three genes gradually decreased, while there was no obvious change of expression of *Mg4* (MGT6 MRS2-4 magnesium transporter 6). *Zn1* (ZIP11 zinc transporter 11) in 70–100 days expression also declined (Fig. 4).

Discussion

Metal nutrients, such as K, Ca, Na, Mg, Fe, Zn, Cu, Mn and Ni, are essential for normal plant growth. Our

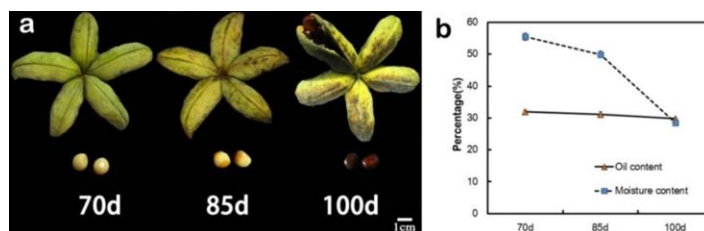


Fig. 1: Seed development and oil content at 70 d, 85 d and 100 d. **a.** Pods gradually turned yellow and cracking at 100 d (Seeds were full maturity). The color of seed skin changed from yellow to brown. **b.** Seeds moisture and oil content from 70 d to 100 d

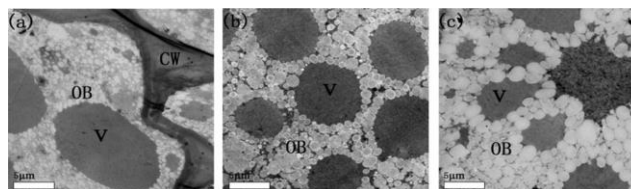


Fig. 2: The development of oil and protein bodies in different periods. **a.** Oil body 70 d is still in the state of small volume, protein body has been formed; **b.** In 85 d, the volume of oil body were increasing; **c.** In 100 d, volume of vacuoles decreased, volume of oil body was increased. OB: oil body, V: vacuoles, CW: cell wall

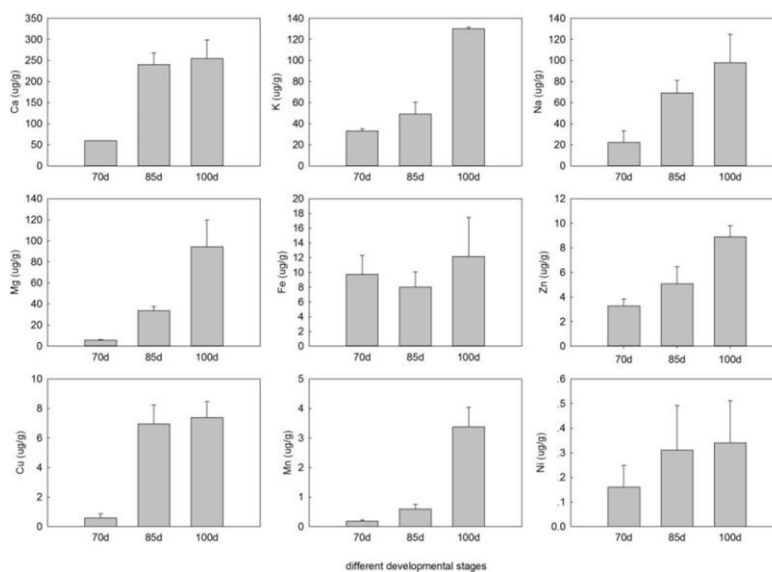


Fig. 3: Measurement of different mineral content across the developmental period of *Paeonia ostii* seed. 70 d: 70 days after anthesis, 85 d: 85 days after anthesis, 100 d: 100 days after anthesis

results showed that the mineral content in *P. ostii* seeds oil was gradually accumulated until maturity stage. According to the suggestion of WHO and the dietary guidelines for Americans, daily intake of Na and K for adult should be 5 g and 4.7 g with Na/K ratio of 1.06. The Na/K ratio in *P. ostii* seeds oil is 0.75 close to the recommend one (Jiang *et al.*, 2013). Large numbers of animal and clinical studies have been certified that Na intake too much can not only increase the kidney and heart load caused by vascular arterial blood pressure, but also can cause tissue damage of kidney and vascular (Kuschvihrog *et al.*, 2015).

High potassium (K) diet can keep low blood pressure, but also may have a direct protective effect on tissues and organs (Wang *et al.*, 2005).

Most of the current studies focused on organic ingredients of plant oil, few study pay close attention to mineral components. This study showed that mineral elements in *P. ostii* seed oil reached a peak value at maturity stage. Among these mineral elements, the content of Ca element was the highest and Ni content as the lowest. To understand the difference of mineral content, we carried out an investigation and comparison between *P. ostii* seed oil and peanut oil, soybean oil and

Table 1: Primers for qRT-PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Ubiquitin	GACCTATACCAAGCCGAAG	CGTTCAGCACCAATC
qCa1	ACTATCTTGAGATCTTCTACTGCAC	GAGACAATACTGGCAAAGACATCGG
qCa2	TCCAAAGGACAAAAGCCACATACTC	TGGGCAAGGAAAAGCATGTACTCGT
qCa3	ATTTACACACACTCTTACCCACTCC	AACTGTCTAACTGGTTTACTGTGAA
qFe1	AGATAAAACTGGAACCTCTGGACCTA	TTTCTCCACCTTGTCTACATCCGA
qFe2	TTCTTATCCCATCCCCCTTCCAGTC	ATAAGACAATGGTTCAATCCCTCGT
qFe3	GTCGCTTATTGACCGCTGGAACACC	GTGGCAGCAATCTCATTACATACG
qK1	GAAATGTTCTTATCTTCCGTGGTG	CAGAAGCACAGTGTCCGCACAGTTG
qK2	CTATGTCATCAACGGCAGTGGTC	CTCCTTGAAGAACACCAGAAGTACC
qK3	ACGGCACGGTGAAACGATACGA	GTGATGAAGTGGGAGAAAAATGTGGG
qMg1	GTGATATATGGACTGAGTTGAACCG	CTTCAATACCCTCAACGAACGCAACT
qMg2	CGGAGAGTTCAAAAAGGTCAGGGATG	AACTGGAGCAGAAGCAGATAGCC
qMg3	ATGTCGTCAGCTCAAAGATTCCG	CAAATCTTAGAATCGTGAAGCAATG
qMg4	GAGATGCTTACCCTGTATTGGACGAAC	ATTCAAACCCCGGCCAACATTAGT
qZn1	CTAAGGGATGCCGATTGGGTGTA	AACTGCAATTACCCCACTCCAAGA
qZn2	CACCATTGCCACAAAACCCAGTAAAC	ATACAGACTAGGCGCCAGTGCAATT

Table 2: Annotation of gene function

Gene name	Gene ID	Contiglength (bp)	BlastX hit	Query cover (%)	Identity (%)	<i>Arabidopsis</i> homolog	Functional annotation
Ca1	comp78393	1758	XP_013451524.1	86	67	AT5G17860.1	CAX7 calcium exchanger 7
Ca2	comp85382	465	XP_007021642.1	70	36	AT1G53210.1	Symbols_sodium/calcium_exchanger_family_protein
Ca3	comp29771	966	XP_007028936.1	100	91	AT5G06850.1	C2 calcium/lipid-binding plant phosphoribosyltransferase
Fe1	comp23550	483	XP_010099080.1	94	74	AT4G33910.1	(2OG) and Fe(II)-dependent oxygenase
Fe2	comp34600	1461	XP_007046709.1	91	67	AT4G36090.3	Oxidoreductase_2OG-Fe(II)_oxygenase_family
Fe3	comp46140	1158	ETK95680.1	95	99	AT2G30390.1	FC2 FC-II, ATFC-II ferrochelatase_2
K1	comp1897982	540	KUG00624.1	100	100	AT4G32500.1	AKT5 KT5 K+ transporter 5
K2	comp42250	1791	XP_007012870.1	92	88	AT2G19600.1	ATKEA4_KEA4_K+_efflux_antiporter
K3	comp62460	2364	XP_006368215.1	99	89	AT2G35060.1	KUP11_K+_uptake_permease
Mg1	comp24953	1374	XP_008901289.1	100	99	AT2G04305.1	Magnesium_transporter_CorA-like_family
Mg2	comp39877	1368	XP_007023461.1	97	92	AT1G16010.3	MGT2_magnesium_transporter_2
Mg3	comp66975	1131	XP_010240915.1	81	69	AT1G61790.1	Oligosaccharyltransferase_complex/magnesium_transporter_family
Mg4	comp78471	1374	XP_003629954.1	83	76	AT3G58970.1	MGT6_MRS2-4_magnesium_transporter_6
Zn1	comp61770	963	XP_010106522.1	83	58	AT1G55910.1	ZIP11_zinc_transporter_11

Table 3: Different varieties of metal elements in the plant oil measurement results

Sample	<i>Paeonia ostii</i> seeds oil	Soybean oil	Peanut oil	Sunflower seed oil
Ca	254.45±25.59	276.32±2.08	380.26±4.82	285.91±1.58
K	130.15±0.94	32.23±0.00	28.90±0.00	30.80±0.65
Na	97.95±0.15	26.48±0.31	39.10±0.49	34.22±0.72
Mg	94.46±14.56	71.48±1.06	100.86±0.53	68.86±0.96
Fe	12.16±3.06	68.59±0.54	61.95±0.56	58.66±0.93
Zn	8.89±0.53	1.66±0.055	3.80±0.069	3.32±0.10
Cu	7.39±0.62	1.97±0.069	1.44±0.050	1.44±0.00
Mn	3.38±0.38	ND	ND	ND
Ni	0.34±0.10	ND	ND	ND

sunflower oil (Jiang *et al.*, 2013). The results showed that among the four plant oil, the highest Ca concentrations was in peanut oil 380.26 µg/g, followed by sunflower oil with 285.91 µg/g and soybean oil of 276.32 µg/g, while in *P. ostii* seed oil content of Ca was 254.45 µg/g as the lowest (Table 3). Interestingly, the K concentration of *P. ostii* seed oil was the highest (130.15 µg/g) and 4 to 4.5 times more than the other three plant oil, the K concentration of peanut oil was 28.90 µg/g as the lowest. Similar to K, Na content in peony seed oil was also the highest which reached 97.95 µg/g, while the lowest was for soybean 26.48 µg/g. Compared to Mg content (100.86

µg/g) in peanut oil, the concentration of Mg in *P. ostii* seed oil ranked the second (94.46 µg/g). The Fe content of *P. ostii* seed oil was the lowest and reached to 12.16 µg/g. It is notable that, Zn content of *P. ostii* seed oil ranked the highest, reaching 8.89 µg/g, while the lowest from soybean oil was only 1.66 µg/g. As a plant that can tolerate copper (Cu), *P. ostii* may have different regulatory mechanisms in dealing with environmental Cu stress. The Cu mineral concentration (7.39 µg/g) in *P. ostii* seed oil was also the highest among the four, which may be associated with *P. ostii* to grow well in Cu mine. Among the four plant oils, Mn and Ni elements were only detected in *P. ostii*, and Mn

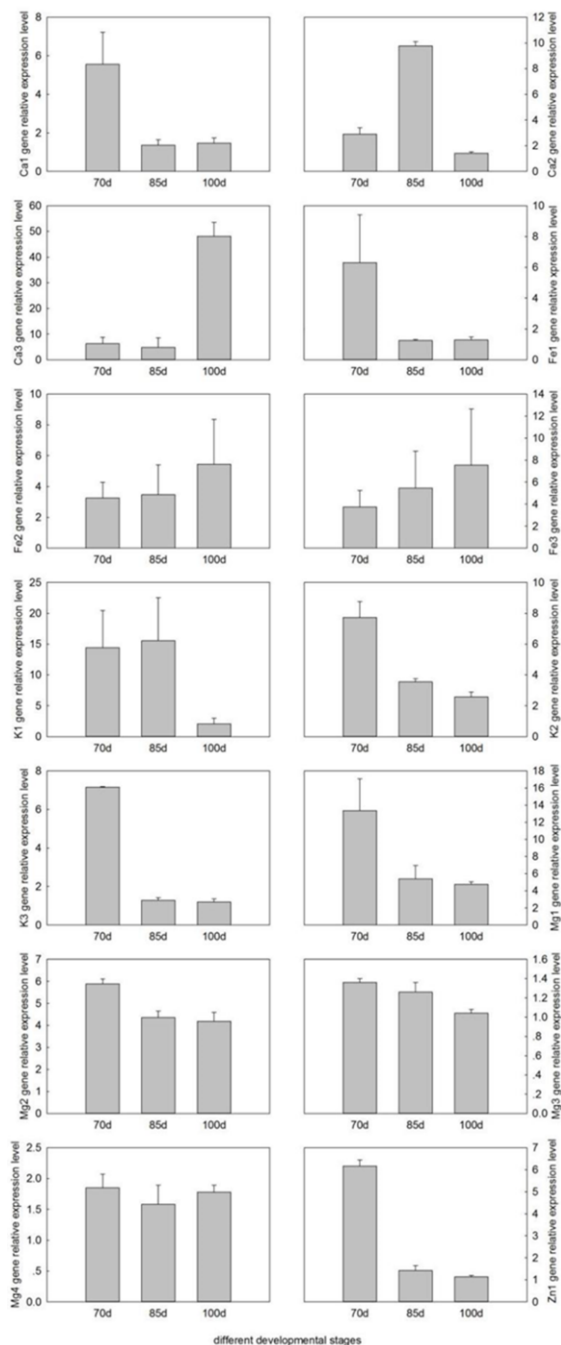


Fig. 4: Expression analysis of seed development related genes in three different petals of '*Paeonia ostii*'. 70 d: 70 days after anthesis, 85 d: 85 days after anthesis, 100 d: 100 days after anthesis

content reached 3.38 $\mu\text{g/g}$ with the Ni contents of 0.34 $\mu\text{g/g}$ (Table 3).

In previous studies, all sorts of proteins specific for metals have been defined and characterized in plants, which including secondary transporters and transport systems comprising pumps that transfer ions across the cellular membranes. Among them, Ca^{2+} /cation antiporter (CaCA)

family make up integral membrane proteins that transport Ca^{2+} can be categorized into five major families: NCX, NCKX and CCX, YRBG, CAXs (Pittman and Hirschi, 2016). Previous studies showed that there are 11 CAX genes (*AtCAX1-11*) in the genome of *Arabidopsis thaliana* (Shigaki and Hirschi, 2006). In present study, *PoCa1* showed decreasing expression during 70 d to 100 d. *PoCa1* was chosen as homologous gene of *AtCAX7* Ca exchanger 7, which indicated that *PoCa1* constituted the potential component of Ca arrangement in plant. *PoCa2* was chosen as Na/Ca exchanger family protein. The expression of *PoCa2* in seeds increased from 70 d to 85 d, then declined at 100 d. *PoCa3* was chosen as calcium/lipid binding plant phosphoribosyltransferase. The sharp raised expression of *PoCa3* from 85 d to 100 d accompanied by volume and increased number of oil body. Recently, biochemical investigations demonstrated that the expression of caleosins (calcium-binding proteins) was a component of oil body membranes during seed germination (Shigaki and Hirschi, 2006). Its role is mainly manifested as example, Fe can be easy to reduce or oxidize in biochemical reactions and making it well fit for its character in redox active proteins to participate in respiration, nitrogen fixation and photosynthesis. Fe is an indispensable micronutrients for a strong and healthy body and one of the constituents forming blood hemoglobin (Rasouli and Ghavami, 2016). Zinc (Zn) is a crucial micronutrient for plants; it also can get toxic at elevated concentrations. Transmembrane Zn transport proteins and Zn chelators took part in avoiding its toxic effects (Ricachenevsky *et al.*, 2015). In our results, the expression of *Zn1* was similar with *AtZIP11* which down regulated during three stages. However, it was reported that *AtZIP11* had the capacity to supplement the Zn-uptake defective yeast mutant *zrt1zrt2* (Milner *et al.*, 2013).

Several studies revealed the molecules involved in metal acquisition in roots, distribution in the plant and transportation to seeds (Zhang *et al.*, 2015). In the living systems, most metal ions were found have structural and catalytic roles in some biological functions.

For example, plants that gather and tolerate a quantity of Zn and grow ample biomass can be useful for phytoremediation, permitting removing dirt from soil contaminated with metals (Vamerali *et al.*, 2010). The study of Zn hyperaccumulators can provide signs useful for biofortification, for the development of crop with a lot of nutrients in the edible tissues. It needs to concentrate on the functional characterization of Zn transporters in planta, sensing mechanism and instruction of Zn uptake, and upon understanding the babble between physiological processes and Zn homeostasis (Milner *et al.*, 2013). These are proves that the importance of molecules to participate in metal acquisition in seed. The current studies concern about the common use of products, which containing mineral oil have grown up as these compounds are detected in all kinds of foods and organic components of edible vegetable oil (Milner *et al.*, 2013). There are few studies about mineral

elements in plant seeds oil, therefore the future research should focus on the metal components of the plant seeds oil. It is a reference value involving the plant seeds oil content that should be discussed for getting a feasible solution to a lot of various food categories in low fat contents.

Conclusion

Number and size of oil bodies in *P. ostii* seed kept growing during seed maturation process. Concentration of mineral elements in *P. ostii* seed oil keeps accumulating during development process and reaches stable state during the seed maturing stage. Tendency of 14 transport genes related to mineral elements varies during seed maturation process, thus providing theoretical basis for further research of *P. ostii* seed oil mineral elements.

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