Effects of Phosphorus Fertilization on Growth Characteristics, Fatty Acid Composition, and Seed Yields of Fengdan (Paeonia ostii T. Hong & J. X. Zhang)

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Abstract. Paeonia ostii T. Hong & J. X. Zhang is a perennial oil and medicinal plant with great importance as well as landscaping. P. ostii is being extensively planted in China, but the soil fertility limits the yield and quality. There is little information available on the effects of phosphorus fertilization on productivity, physiological characteristics, and seed yield and quality. This study investigated the effects of phosphorus fertilization on productivity, physiological characteristics, and seed yield and quality. This study investigated the influence of different phosphorus levels, 0 kg·hm$^{-2}$ (CK), 90 kg·hm$^{-2}$ (P1), 135 kg·hm$^{-2}$ (P2), 180 kg·hm$^{-2}$ (P3), 225 kg·hm$^{-2}$ (P4), and 270 kg·hm$^{-2}$ (P5), on the photosynthesis, morphology, physiological parameters, and yield of P. ostii. The results indicated that the net photosynthetic rate, stomatal conductance ($g_s$), and transpiration rate of P. ostii increased significantly with the application of P4, which increased by 34.77%, 65.72%, and 21.00% compared with CK, respectively. Simultaneously, the contents of soluble sugar, soluble protein, and photosynthetic pigment in P4 were the highest compared with other treatments. In addition, thousand-grain weight (326.4 g) and seed yield per plant (37.33 g) of P4 were significantly higher than the control. However, the total amount of unsaturated fatty acids in P4 was lower compared with other treatments. The indexes of high correlation coefficients with Dim 1 and Dim 2 were $g_s$ and superoxide dismutase (SOD), respectively. The results showed that phosphorus levels improved plant photosynthetic capacity and increased antioxidant capacity as well as seed yield. Furthermore, phosphate fertilizer had significant effects on the oil composition. Moreover, the effect of phosphorus application rate on the growth index of P. ostii was greater than that of the physiological index.

Tree peony (Paeonia section Moutan DC.) is a perennial deciduous shrub in the family Paeoniaceae, genus Paeonia, with high ornamental and medicinal values (Guo et al., 2017; Li et al., 2015; Zhang et al., 2019; Zhao et al., 2015). It is endemic to China and has an ornamental cultivation history of more than 2000 years (Cui et al., 2016). Among the nine species of tree peony, Paeonia ostii T. Hong & J. X. Zhang was predominantly approved as a new oil-use resource by the China State Food and Drug Administration in 2011 as strong seed setting capacity and high oil yield (>22%) (Liu et al., 2020). P. ostii has a star-shaped fruit with dark oval seeds characterized by abundant unsaturated fatty acids (>90%) and a high proportion of n-3 fatty acids, which can prevent many diseases (Wang et al., 2019; Wei et al., 2016; Zhang et al., 2016). The unsaturated fatty acid content in P. ostii seed oil complies with the international nutritional standards of “omega meals,” and is similar to oil from olive and camellia, which are among the most popularized and healthiest cooking oils in the world (Liang et al., 2017).

Phosphorus plays an important role in crop growth, yield, and quality formation. The availability of phosphorus controls crop growth and development (Wyngaard et al., 2016). As one of the three essential elements of plants, second only to nitrogen, phosphorus is a major component in materials such as nucleic acids, phospholipids, high-energy phosphate bond compounds, and various coenzymes (Wyngaard et al., 2016). Phosphorus plays an important role in carbohydrate and nitrogen metabolism and in the mutual transformation of protein metabolism and carbohydrates (Yao et al., 2012). However, 40% of the world’s arable land for crop production is limited by phosphorus. Therefore, it has been found to be a major limiting nutrient for crop growth in many agroecosystems and it is indispensable in several physiological and biochemical processes (Simpson et al., 2011). Studies have shown that phosphorus can increase the yield of grain crops by ≈10% to 250%, increasing the economic yield and quality of crops such as cotton and tobacco (Chen et al., 2016; Rouached et al., 2010). Phosphorus also aids in root development, flower initiation, and development of seed and fruit (Wei et al., 2016). Moreover, plant demand for phosphorus varies with plant and soil fertility. The effects of different phosphorus fertilizer application rates on plants are different. When the amount of phosphorus was optimal, it could promote chlorophyll content, soluble sugar content, and various protective enzyme activities (Ma et al., 2017). On the contrary, when the content is excessive, phosphorus will adversely affect enzyme activity and reduce the content of soluble sugar and chlorophyll (Ma et al., 2017), resulting in a decrease in photosynthesis (Singh et al., 2016b). When phosphorus is deficient, plant growth will be inhibited and red patches will appear on the leaves. Moreover, plant height, root length, number of branches and roots, leaf area, and plant photosynthetic capacity will be reduced (Ohdo and Takahashi, 2020). Furthermore, excessive phosphorus leaching will lead to eutrophication and environmental pollution.

Phosphorus nutrition is the main limiting factor affecting agricultural high yield in China. To a large extent, the application of chemical fertilizer is a very effective method to improve crop yield. This is true for many different crops that grow under the most widely changing conditions around the world (Gendy et al., 2015). Numerous field experiments have shown that chemical fertilization is the most critical growth-limiting factor (Gendy et al., 2013). However, as a new type of oil crop, the research on the effects of phosphorus nutrition on the growth characteristics, fatty acid composition, and seed yields of P. ostii is relatively rare. Therefore, the single-factor control experiment was used to explore the effect of phosphorus fertilizer on the yield and quality of peony seeds. The experiment laid a foundation for determining the local fertilization formula. On the premise...
of avoiding excessive phosphorus fertilizer, the purpose of high yield can be achieved.

**Material and Methods**

**Plant material and growing conditions.** The study was carried out in the oil peony plantation of Yibin District (lat. 34°39'55.43" N, long. 112°36'19.65" E), Luoyang City, Henan Province, China, which has a temperate semihumid and semiarid continental monsoon climate (Wang et al., 2019). The annual average temperature was 12.1 to 14.6 °C during the trial period. The precipitation and sunshine in the test site were abundant, with an annual average precipitation of 600 mm and a total of annual sunshine hours ranging from 2300 to 2600 (Wang et al., 2019). The study site had an alluvial soil type, containing 14.93 g·kg⁻¹ of organic matter, 0.87 g·kg⁻¹ total N, 13.96 mg·kg⁻¹ alkali nitrogen, and 15.265 mg·kg⁻¹ quick-acting potassium at the beginning of the experiment. The pH was 7.68. By observing and recording the plant growth before the experiment, there was an obvious lack of soil fertility. The land was relatively barren. Field management measures included weeding, irrigation, and application of urea (180 kg·hm⁻²) and potassium chloride (225 kg·hm⁻²).

*P. ostii* were 3-year-old seedlings transplanted in 2013. The average plant height was 74.9 cm in 2016. The experiment used a single-factor random block design. Plants were randomly grouped for control (CK, 0 kg·hm⁻²) and five phosphorus fertilizer treatments (P1, 90 kg·hm⁻²; P2, 135 kg·hm⁻²; P3, 180 kg·hm⁻²; P4, 225 kg·hm⁻²; P5, 270 kg·hm⁻²). CK indicated water treatment without adding phosphorus fertilizer. The study was carried out in three different 20 m² plots with each plot containing 45 plants for each treatment. Planting density was 60 × 75 cm. The phosphate fertilizer was calcium superphosphate. In this experiment, phosphorus fertilizer was applied before and after flowering, and the proportion of fertilization experience was 7:3 according to the local reality.

**Determination of leaf photosynthesis.** At the flowering stage, various photosynthetic parameters of *P. ostii* leaves were measured with a Li-6400 photosynthesis system (LI-COR, Inc., Lincoln, NE) from 9:00 to 11:00 AM on 22 Apr. 2016. Three individual plants with a similar growing status were selected from each treatment. Net photosynthetic rate (Pn), gₛ, intercellular carbon dioxide concentration (Ci), and the transpiration rate (Tr) of *P. ostii* under different phosphorus treatments. There was no significant difference in the mean value represented by the same letter at the level of 5% using the Duncan method. CK, without phosphorus treatment.

**Fig. 1.** The net photosynthetic rate (Pn), the stomatal conductance (gₛ), changes of intercellular carbon dioxide concentration (Ci), and the transpiration rate (Tr) of *P. ostii* under different phosphorus treatments. There was no significant difference in the mean value represented by the same letter at the level of 5% using the Duncan method. CK, without phosphorus treatment.

**Fig. 2.** The major fluorescence parameters of *P. ostii* leaves under different phosphorus treatments. Fo, minimal fluorescence; Fv, variable fluorescence; Fv/Fm, the maximum photochemical efficiency; ØPSII, the actual photochemical efficiency. There was no significant difference in the mean value represented by the same letter at the level of 5% using the Duncan method. CK, without phosphorus treatment.
Determination of key physicochemical indices. Leaves were sampled and pooled at the flowering stage based on treatment types and different plots. The activity of SOD, peroxidase (POD), and catalase (CAT) was determined by the nitroblue tetrazolium (NBT) assay, the guaiacol method, and ultraviolet absorption, respectively (Zhang et al., 2014). Malondialdehyde (MDA) was measured by using thiobarbituric acid (Zhang et al., 2015a). Soluble sugar content was estimated by anthracene ketone-sulfuric acid colorimetry. Soluble protein content was determined by using Coomassie brilliant blue G-250, and chlorophyll content was measured by the Lichtenthaler and Wellburn method (Sa et al., 2013).

Harvests. When the fruit matured, 10 individuals with stable growth were selected from each treatment, and the length and width of the fruit were measured with a vernier caliper with an accuracy of 0.1. Then, the 1000 grain weight and the yield per plant were measured with an accuracy of 0.01.

Extraction of \textit{P. ostii} seed oil and oil yield. To preserve the active substances, the \textit{P. ostii} seed oil was prepared by supercritical extraction (Reverchon and De Marco, 2006). Three hundred grams of seeds from each treatment were desquamated, crushed, and filtered through a 100-mesh screen. In consideration of the lighter color of \textit{P. ostii} seed oil, the degumming and decoloring procedure was cancelled. The extraction was carried out under the conditions of 30 L·h$^{-1}$ traffic volume, 35$^\circ$C and 30 MPa pressure time, after 60 min, and the extraction efficiency reaches 98.2% (Liu et al., 2020).

Analysis of fatty acid compositions in \textit{P. ostii} seed oil. The gas chromatography–mass spectrometry (GC-MS) method was used to analyze the fatty acid component of seed oil. Under the same conditions, each sample was tested three times. The column was a TG-5MS (30.0 m × 0.25 mm, 0.25 μm) elastic quartz capillary column. The carrier gas was high-purity helium (99.999%) with a flow rate of 5 mL·min$^{-1}$. The injection mode was 80:1 shunting sample injection with the injection volume of 0.2 μL, and the injection temperature was 260°C. The column flow rate was 5.0 mL·min$^{-1}$, and the GC-MS interface temperature was 250°C. The ion source was electron ionization, and the ion source temperature was 250°C. The temperature of transmission line was 250°C. The power voltage was 70 eV, and the scan range (m/z) was 30 to 460 amu, with a scan time of 0.2 seconds.

Statistical analysis. The data were statistically analyzed by R software (R Core Team, 2019). The significance data ($P < 0.05$) were analyzed by multiple comparison range test to analyze the impact of phosphorus application on each index. Principal component analysis (PCA) and redundancy analysis (RDA) were used to analyze the importance of phosphorus fertilizer on the physiology and growth index of \textit{P. ostii}. In our analysis, variance partitioning led to three individual fractions: 1) unique physiological index; 2) unique growth index; as

![Fig. 3. Physiological indexes of \textit{P. ostii} under different phosphorus treatments. There was no significant difference in the mean value represented by the same letter at the level of 5% using the Duncan method. CK, without phosphorus treatment.](image)

![Fig. 4. Photosynthetic pigment content of \textit{P. ostii} leaves under different phosphorus treatments. There was no significant difference in the mean value represented by the same letter at the level of 5% using the Duncan method. CK, without phosphorus treatment.](image)
Results

Net photosynthetic rate, gs, intercellular CO₂ concentration and transpiration rate at different phosphorus levels. The net photosynthetic rate showed a single peak trend with the increase of phosphorus application, and P4 had the highest net photosynthetic rate. Compared with the control, the net photosynthesis of P1 to P5 treatment increased by 0.60%, 18.48%, 21.24%, 35.82%, and 25.51%, respectively (Fig. 1), indicating that the photosynthesis of P. ostii responded to the phosphorus application. Phosphorus application had significant effects on gs (P < 0.05). As shown in Fig. 1, gs exhibited a similar trend as photosynthesis. Compared with the control, the gs of P1 to P5 treatments increased by 11.63%, 15.01%, 31.97%, 65.72%, and 29.82%, respectively. Under different phosphorus treatments, there were significant differences in intercellular CO₂ concentration at flowering stage of P. ostii (P < 0.05), and with the increase of phosphorus application, the intercellular CO₂ concentration decreased until P4 (Fig. 1). The result showed that phosphorus application significantly reduces the intercellular CO₂ concentration. Compared with the control, P1 to P5 treatments decreased CO₂ concentration by 2.26%, 5.89%, 6.63%, 12.93%, and 6.87% (Fig. 1), respectively. During flowering stage, with the increasing of phosphorus application, the trend of change of intercellular CO₂ concentration was opposite to that of the net photosynthesis rate. Under different phosphorus treatments, there were significant differences in the transpiration rate at flowering stage of P. ostii (P < 0.05), and with the increase of phosphorus application, the transpiration rate increased until P4 (Fig. 1). Compared with the control, P1 to P5 treatments increased the transpiration rate by 4.00%, 6.69%, 11.84%, 21.00%, and 6.29% (Fig. 1), respectively.

Chlorophyll fluorescence parameters under different phosphorus levels. Phosphorus application had significant effects on Fv, Fv/Fm, and ØPSII. Fv/Fm and ØPSII of P4 were the largest, 0.79 and 0.31, respectively, whereas Fv/Fm and ØPSII of CK were the lowest. Compared with the control, Fv, Fv/Fm, and ØPSII of P4 increased by 14%, 18%, and 63%, respectively (Fig. 2).

Physicochemical parameters under different phosphorus levels. Phosphorus application had significant effects on CAT, soluble protein, soluble sugar, and MDA at flowering stage (Fig. 3). CAT, soluble protein, soluble sugar, and MDA (including CK) increased with the application of P4 compared with P5, and there were significant differences. Thus, CAT, soluble protein, and soluble sugar increased 65%, 22%, and 68%, respectively, in P4 compared with lower phosphorus application (CK). MDA content in P4 was reduced by 46% compared with CK. However, phosphorus application has little effect on SOD and POD. Phosphorus application had significant effects on chlorophyll a, chlorophyll b, and chlorophyll content (Fig. 4). However, there were no differences among phosphorus treatments for chlorophyll a/b (averaged in 3.67) and carotenoid (averaged in 0.26 mg L⁻¹). The average values of chlorophyll a, chlorophyll b, and chlorophyll content were 1.16 mg L⁻¹, 0.32 mg L⁻¹, and 1.48 mg L⁻¹, respectively.

Morphological parameters under different phosphorus levels. There were no differences among phosphorus treatments for crown width (averaged in 85.47 cm) and leaf width (averaged in 12.36 cm) (Fig. 5). Compared with the control, P4 significantly increased plant height, leaf length, number of new branches, and flower diameter. Content of main fatty acids under different phosphorus levels. Phosphorus application had significant effects on the fatty acid content of seed oil. With the increase of phosphorus application, the total amount of saturated fatty acids increased, while the total amount of unsaturated fatty acids decreased (Fig. 6). The stearic acid content and α-linolenic acid of P4 increased by 53% and 8% compared with CK, respectively.

Differences of seed yields under different phosphorus levels. Phosphorus application had significant impacts on the fruit pod, fruit pod length, fruit pod diameter, seed yield, and 1000 grain weight. The fruit pod, fruit pod length, fruit pod diameter, seed yield, and 1000 grain weight (including CK) increased with the application of P4 compared with P5, and there were significant differences. Thus, the fruit pod, fruit pod length, fruit pod diameter, seed yield, and 1000 grain weight increased 43%, 35%, 18%, 39%, and 19%, respectively, in P4 compared with CK (Fig. 7).

PCA analysis and RDA analysis of various indexes of different phosphorus treatments. The collinearity test was carried out for all indicators to remove the indicators with high correlation. The first principal component clearly distinguished P4 phosphorus application from other treatments, and explained 39.3%. The correlation coefficients of gs, gS, Tr, seed grain, and Fv/Fm with the first principal component were 0.51, 0.42, 0.38, and 0.38, respectively. The correlation coefficients between SOD and seed grain and the second principal component are −0.70 and 0.51, respectively (Fig. 8). The change of phosphorus application rate on all indexes of P. ostii explained 92% (physiological index 80%; growth index 87%), and the interaction of physiological index and growth index had a higher response to phosphorus application rate (75%) (Fig. 9).

Discussion

The application of phosphorus fertilizer significantly affects the photosynthetic
capacity of *P. ostii* (Fig. 1), because in photosynthesis, phosphorus can promote photosynthetic phosphorylation, produce a large amount of ATP, reduce the heat dissipation of light energy in plant leaves and improve the utilization capacity of light energy, so as to promote photosynthesis and improve the synthesis of carbon hydrate (Ikhajiagbe et al., 2020). According to the PCA, the correlation coefficient between $g_S$ and transpiration rate and Dim1 is high (Fig. 8). It is reported that during filling stage, an appropriate amount of phosphorus can promote leaf $g_S$, transpiration rate, and reduce CO$_2$ concentration in the environment, so as to improve the net photosynthetic rate (Li et al., 2010). Herein, P4 treatment significantly increased $g_S$ and transpiration rate, reduced intercellular CO$_2$ concentration, and improved photosynthetic capacity (Fig. 2). This is consistent with the results of previous studies. Moreover, the maximum photochemical quantum efficiency (refers to the energy capture efficiency of an open PSII reaction center) and $\Phi_{PSII}$ (the photochemical quantum efficiency of the actual PSII in the presence of applied light) are higher at the P4 level (Fig. 2), indicating that P4 treatment has higher light utilization efficiency, so as to improve the ability of photosynthesis (Hazrati et al., 2016). In addition, the application of appropriate amount of phosphorus can effectively promote the growth and development of vegetative organs. It can also increase photosynthetic pigments (Fig. 4) and osmotic regulators to a certain extent and delay the aging of photosynthetic organs (Fig. 3) (Wang et al., 2016b). As an indicator of lipid peroxidation of cell membrane, MDA is not only a peroxide product, but also reacts with other chemicals in cells, causing severe damage to the cell membrane system. The significant increase of MDA indicates rapid decay of plants, and plant senescence is closely related to active oxygen metabolism (Wu et al., 2010). SOD, CAT, and POD are important protective enzymes of active oxygen scavenging enzyme system. They can effectively prevent the accumulation of high concentration oxygen, prevent membrane lipid peroxidation, delay plant aging, and maintain normal plant growth and development (Yang and Gao, 2001). In this study, the improvement of photosynthetic capacity of P4 treatment significantly increased the accumulation of plant nutrients, the content of soluble sugar and soluble protein, and the adaptability to the external environment, resulting in the significant improvement of SOD, POD, and CAT in leaves compared with the control (Fig. 3).

An appropriate phosphorus application rate had a positive effect on plant growth parameters (Chrysargyris et al., 2016). Compared with the control, the plant height, leaf length, number of new branches, and flower diameter of P4 were significantly increased (Fig. 5). Previous studies showed that the plant height of *Camellia oleifera* increased with the addition of phosphorus application (Luo et al., 2016). Phosphorus fertilizer had different effects on the growth and development of lavender, lettuce, and rice, and...
promoted the crown width, leaf length, and new branches of lavender (Chrysargyris et al., 2016). The promoting effect on lettuce growth is mainly reflected in plant height, dry matter, and leaf number under two soil bulk densities (Azzi et al., 2017) and increasing rice leaf length (Guo et al., 2012). The effects of phosphorus fertilizer on crops are also different in different periods. There was no significant effect on seedling stage. However, the application of phosphorus fertilizer in fruit stage significantly promoted the plant height and crown growth of pepper (Wang et al., 2016a).

There are many problems in the production of oil peony, such as excessive use of fertilizer and unbalanced application of nitrogen, phosphorus, and potassium fertilizer, which seriously affect the fertilization effect and farmland environmental quality (Zhang et al., 2018). Excessive application of phosphorus not only inhibited leaf growth, but also reduced the activity of protective enzymes in leaves. Thus, the structure and function of photosynthetic organs are destroyed by reactive oxygen species, and the aging process is accelerated, resulting in lower leaf fluorescence and transpiration rate (Wang et al., 2010; Wang et al., 2016b; Zhao et al., 2008; Zhao et al., 2009), which leads to the reduction of photosynthetic rate and photosynthetic products of *P. ostii*. Compared with P4, SOD, POD, and CAT activities were decreased under low (CK) and high phosphorus (P5) (Fig. 3). It indicated that under the stress of low and high phosphorus, the physiological metabolism of *P. ostii* was inhibited, and the activities of SOD, POD, and CAT could not be maintained at a high level. The content of MDA in *P. ostii* increased with the application of P5 compared with P4, and there were significant differences, which indicated that excessive phosphorus caused the accumulation of reactive oxygen species and significantly increased the membrane lipid peroxidation (Lian et al., 2015).

Excessive application of phosphorus fertilizer leads to the reduction of plant phosphorus utilization rate and the accumulation of plant dry matter, which is not conducive to plant growth. For example, with the increase of phosphorus fertilizer, the plant height of camellia decreased after exceeding the threshold (Luo et al., 2016). Excessive application of phosphorus fertilizer inhibited the growth of plant height and crown width of pepper (Wang et al., 2016a).

It was reported that phosphorus application significantly affected the oil content and composition of *Origanum dictamnus* seeds (Anastasia et al., 2002) and significantly improved the oil content of basil seeds (Ramezani et al., 2009). The results of this study showed that the phosphorus application significantly affected the ratio of saturated fatty acids to unsaturated fatty acids in *P. ostii* (Fig. 6). The unsaturated fatty acid content of *P. ostii* seed oil can reach 93.04%, which is much higher than the existing high-quality vegetable oils, such as olive oil and flax oil (Tang et al., 2013). However, with the increase of the amount of phosphate fertilizer, the content of total unsaturated fatty acids decreased, which may be due to the role of lipooxygenase, free radicals, and reactive oxygen species (Xu and Zou, 1993). Previous reports also showed that compared with other vegetable oil varieties on the market, the content of unsaturated fatty acids beneficial to human body in *P. ostii* seed oil was higher (Liu et al., 2020). Therefore, rich α-linolenic acid in *P. ostii* seed oil has attracted more and more attention.

Phosphorus plays an important role in increasing crop yield, which can significantly increase the number of grains per panicle and 1000 grain weight (Xing et al., 2015). In addition, the dry matter accumulation and yield of wheat were significantly improved (Xing et al., 2015). This was consistent with the results of this study. The results showed that phosphorus nutrition levels significantly increased the grain yield and 1000 grain weight of *P. ostii* (Fig. 7). According to the PCA, the correlation coefficient between grain yield and Dim 2 was high (Fig. 8).
Excessive use of phosphorus fertilizer not only wasted phosphorus resources and polluted the environment (Conley et al., 2009), but also reduced grain quality (Zhang et al., 2015b). RDA analysis showed that the effect of phosphorus fertilizer on the growth and physiology of *P. ostii* was important, which explained 92% of the variation (Fig. 9). Therefore, while avoiding excessive application of phosphorus fertilizer, sufficient soil phosphorus should be provided according to the physiological development characteristics of crops to achieve high yield.

**Conclusion**

The effects of different phosphorus applications on the growth and development of *P. ostii* were studied, including plant growth, photosynthetic efficiency, antioxidant activity, grain yield, and seed oil composition. The results showed that P4 had higher chlorophyll content, maximum photochemical rate, and net photosynthetic rate, resulting in the best photosynthetic capacity and grain yield. Moreover, phosphorus application had significant effects on stearic acid and 

**Literature Cited**


