Stimulatory Effect of Fe₃O₄ Nanoparticles on the Growth and Yield of *Pseudostellaria heterophylla* via Improved Photosynthetic Performance

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Abstract. Nanomaterials have recently been used as growth stimulants to promote the production of crops in saline-alkali through root application. However, if applied through leaves, little is known about the effect of Fe₃O₄ nanoparticles (NPs) on the root growth and yield, especially for medicinal crops. To fill this gap, a single factor experiment was conducted to explore the effects of Fe₃O₄ NPs on growth, yield, the dry matter distribution, chlorophyll content, photosynthetic characteristics, chlorophyll fluorescence parameters, and polysaccharide content of *Pseudostellaria heterophylla* by foliar spraying under field conditions. Fe₃O₄ NPs (20–50 mg·L⁻¹) significantly promoted growth, the dry matter distribution of root and root tuber yield per unit area. Fe₃O₄ NPs enhanced net photosynthetic rate (P_n) by increasing chlorophyll content. And Fe₃O₄ NPs increased the daily mean and peak value of P_n , and alleviated the phenomenon of “midday depression” by improving nonstomatal limitation. Chlorophyll fluorescence parameters indicating that Fe₃O₄ NPs promoted the photochemical activity of PSII and alleviated photoinhibition by enhancing the photochemical use of excess excitation energy. Gray correlation analysis showed that Fe₃O₄ NPs enhanced the adaptability of *P. heterophylla* photosynthesis to high temperatures and strong light. Of note, Fe₃O₄ NPs enhanced the polysaccharide content of the root tuber. Phytotoxic effect was recorded at high NPs (100 mg·L⁻¹) doses. Collectively, Fe₃O₄ NPs could promote performance of *P. heterophylla* by improving photosynthetic performance, enhancing its adaptability to the environment, and increasing the distribution ratio of photosynthates to the underground part.

Pseudostellaria heterophylla is a valuable Chinese herbal medicine, used for both medicine and food, that belongs to the *Caryophyllaceae* family (Zhao et al., 2016). *P. heterophylla* is widely used in the traditional and modern pharmaceutical industries (Gu, 2019; Zhang, 2020). The root tubers of *P. heterophylla* contain pharmacological ingredients such as saponins,

polysaccharides, amino acids, and cyclopeptides (Ma et al., 2017a). Modern medical research shows that the polysaccharides in *Pseudostellaria heterophylla* can protect myocardium (Liu and Ruan, 2017), treat diabetes (Chen et al., 2017), and possess antioxidant (Xu et al., 2012). In addition, it can also prevent myocardial infarction and pancreatic cancer (Sun et al., 2020), enhance immune activity, and improve chronic fatigue syndrome (Sheng et al., 2009). In recent years, important medical discoveries about the pharmacological effects of *P. heterophylla* have also greatly promoted the market demand for this plant, and its application prospects are broad (Li et al., 2016). However, extensive artificial cultivation techniques of *P. heterophylla* result in low yield and declining quality and have seriously restricted its industrial development (Xia and Yang, 2010). Therefore, development of new fertilization methods and crop management techniques is critically important.

Currently, with the rapid development of nanoscience, nanotechnology application

aiming at crop protection and improvements of crop agronomic traits engenders considerable interest (Raliya et al., 2018). Nanomaterials-based fertilizers might have benefits such as crops improvement (Parisi et al., 2015), low eco-toxicity (Shankramma et al., 2015), and target-specific delivery of biomolecules (Wang et al., 2016). Iron oxide nanoparticles are one of the most widely explored and applied nanomaterials because they are environmentally benign, readily available, magnetically sensitive, redox active, and biocompatible (Rui et al., 2016). Iron is a crucial trace element for photosynthetic processes and plant growth. Photosynthesis is the physiological basis of dry matter accumulation and high crop yield (Herrmann et al., 2019). The rates of uptake and biomineralization of NPs in different plants have been reported, as well as their transport and localization in cells (Cai et al., 2020; Li et al., 2018; Wang et al., 2011; Zhu et al., 2008). However, there are only a few works on the use of nanomaterials as fertilizers for plants, and most of them are focused on ideal hydroponic conditions, rather than field conditions.

Nanoparticles have biphasic effects on plant growth and development (Tripathi et al., 2017), depending on their chemical composition, reactivity, mode of application, experimental conditions, size, concentration, as well as plant species (Maswada et al., 2018). Most of the research on iron oxide NPs in plants have centered on the toxicity of nanomaterials (Ding et al., 2019; Ghafarian et al., 2013; Tripathi et al., 2017). The toxicity effects of iron oxide NPs may be associated with their size and concentration. Seed germination and root elongation tests showed that NPs with a size less than 50 nm has no inhibitory effect on plant physiology in the concentration range of 0–2000 mg·L⁻¹ (González-Melendi et al., 2008; Zhu et al., 2008). The toxicity thresholds of iron oxide NPs in different plants are also different (Zu-verza-Mena et al., 2017), but the relevant research is still limited. At present, there are few reports on the beneficial effects of Fe₃O₄ NPs; these have concentrated mainly on iron-deficient plants or plants in a saline-alkali environment (Chouliaras et al., 2004). For example, using Fe₃O₄ NPs treatment has improved maize (*Zea mays*) photosynthesis, has lowered the rate of membrane lipid peroxidation, and has been protective from negative impacts of salinity stress (Mahboobeh et al., 2016). Fe₃O₄ NPs can have a positive effect on the growth of muskmelon (*Cucumis melo*) and the vitamin C (VC) content of fruit (Wang et al., 2019). Notably, Elfeky et al. (2013) have reported that the use of Fe₃O₄ NPs can increase the photosynthetic pigment content, medicinal essential oil content, and dry matter yield of basil (*Ocimum basilicum*) plants. Obviously, the use of iron oxide NPs opens a wide range of possibilities in plant research and agronomy. However, research on the use of iron oxide NPs as a fertilizer in herbs still lags behind. To the best of our knowledge, the application of iron oxide NPs in herbs with roots used to prepare medicines

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is still a scientific issue that has not been explored.

With the aim of developing a technical approach for the agricultural application of economical nano materials, we applied the iron nanoparticle strategy to examine possible improvement of the yield of the medicinal plant *P. heterophylla*. The study was designed with the following objectives 1) to study the effect of different concentrations of Fe₃O₄ NPs on the growth and yield of *P. heterophylla*; 2) to evaluate the effect of different concentrations of nanometer Fe₃O₄ NPs on chlorophyll content, the diurnal photosynthetic variation, and chlorophyll fluorescence parameters of *P. heterophylla*; and 3) to evaluate the effect of different concentrations of Fe₃O₄ NPs on the dry matter distribution and polysaccharide content in roots of *P. heterophylla*.

Materials and Methods

Materials. The Fe₃O₄ NPs of 99.5% purity were purchased from Zebra Biotechnology Inc. (Nanjing, China). According to the data provided by the manufacturer, the NPs are spherical, with an average diameter size of 20 nm. The shape and size were determined by a transmission electron microscope (Tecnai G220 TWIN; FEI, Hillsboro, OR). The *P. heterophylla* seeds used in the experiments were provided by the forest station in Shibing county, Guizhou Province.

Experimental site. The experimental site is located in the Xiashu forest farm of Nanjing Forestry University (lat. 31°59'N, long. 119°12'E), Jiangsu Province, China, and it is characterized by a north subtropical monsoon climate. Annual mean temperature is $\approx 15.2^{\circ}\text{C}$, annual mean precipitation is 1104 mm, mean annual sunshine is 2018 h, and the mean growing season is 229 frost-free days. The soil type is yellow-brown soil, with an organic matter content of 7.39 g·kg⁻¹, with total nitrogen (N) and phosphorus (P) contents of 0.704 and 0.146 g·kg⁻¹, respectively, and available P and potassium (K) contents of 12.5 and 103.7 mg·kg⁻¹, respectively. The soil pH is ≈ 5.5 (Ma et al., 2017a).

Experimental design and treatments. The experiments were conducted from Nov. 2018 to June 2019 using a randomized complete block design. The seedlings of *P. heterophylla* were planted in late Nov. 2018. After ploughing, the ridges (length 9 m, width 100 cm, and height 25 cm) were made. Four furrows of about 12 cm in depth with row spacing of 15 cm were created. Suitable amounts of plant ash and compound fertilizer were applied to the soil (Ma et al., 2017b). *P. heterophylla* seeds were sown evenly to keep the distance between each plant at about 4 cm. The sowing rate was 80 g·m⁻². In the experiment, there were four treatments with three replicates in each treatment. Twelve plots were set up with a plot area of 3 m² (3 m × 1 m). The applications of Fe₃O₄ NPs consisted of four treatments of 0 (deionized water), 20, 50, and 100 mg·L⁻¹ represented by CK, Fe20, Fe50, and Fe100, respectively. The uniform and stable suspension of Fe₃O₄ NPs was prepared by ultrasonic

oscillations. The foliar spraying method was employed in the experiment. About 500 mL of solution per area was applied to the foliage until the solution dripped from the plant. According to the root expansion period of *P. heterophylla*, it had sprayed treatment three times, respectively on 17 Apr., 27 Apr., and 7 May. The treatment was completed by 9:00 AM.

Growth index. The growth index of *P. heterophylla* was determined from Apr. to July 2019, including seedling height, diameter, and biomass. The first measurement was determined 10 d after the first treatment, and then every 10 d for a total of five times. Nine seedlings were selected for each treatment. The length, diameter, and dry weight of the root tuber and the root tuber yield per unit area were measured after harvesting. Sixty root tubers were randomly selected to measure the length, diameter, and dry weight of the root tuber in each treatment, and the mean of values were used as the final result for each treatment.

Seedling height and root tuber length were measured with a straightedge. The diameter of the seedling and root tuber were measured with an electronic vernier caliper. The surface of the seedling and root tuber was washed. Then, each seedling was divided into two parts: shoot (the aboveground part) and root (the underground part). Seedlings and root tubers were placed in an oven at 105°C for 15 min, then dried at 60°C to constant weight. The biomass of each part and the dry weight of each root tuber were weighed using an electronic analytical balance (FA1004C; Shanghai Yueping Scientific Instrument Inc., Shanghai, China). The fresh weight of the roots per unit area was weighed. The relative growth was calculated according to the method of Qiu et al. (2007).

Chlorophyll content. Measurement of chlorophyll content was performed using the SPAD-502 chlorophyll meter (SPAD-502; Konica-Minolta, Tokyo, Japan). Before measurements were taken, the instrument was calibrated by measuring transmission with no leaf inside. The meter can calculate a relative value in soil plant analysis development (SPAD) units, which indicates the amount of chlorophyll present in the leaf. The SPAD value of leaves after the initial treatment for 10 d was determined and measured every 10 d. Using random sampling, 18 leaves were selected in each treatment, and each leaf was repeated three times.

Total chlorophyll (chlorophyll a and b) was measured according to Arnon (1949). One gram of fresh leaves was taken in the middle of the flowering period, ground with 10 ml of 80% acetone, and then centrifuged at 5000 rpm for 5 min. The absorbance of the solution was read at 645 nm and 663 nm against the solvent (acetone) blank using an ultraviolet-160A recording spectrometer (Shimadzu UV180; Shimadzu Corp., Tokyo, Japan).

Dynamic changes of the net photosynthetic rate. The P_n was recorded using the LI-COR 6400 portable photosynthesis system (LI-COR 6400; Li-COR Bio-sciences, Lincoln, NE) at a CO₂ level of 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ and a

light intensity of 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by an LED red/blue light source. P_n was measured between 9:00 AM and 11:00 AM. P_n was performed five times following the first NPs treatment, once every 10 d. Six leaves that were deemed healthy, mature, and displayed a consistent growth state were identified in each treatment and measured.

Diurnal variation in photosynthesis. The diurnal changes in photosynthesis in the leaves of *P. heterophylla* were measured on 17 May (a sunny day) by using a LI-COR 6400 portable photosynthesis system (LI-COR 6400; Li-COR Bio-sciences). Measurements were conducted in 6 cm² leaf chambers in an open gas system with natural light and uncontrolled ambient CO₂ concentration and air temperature. Six leaves that were deemed healthy, mature, and displayed a consistent growth state were identified in each treatment and measured. Measurements were made at 2-hour intervals from 8:00 AM to 6:00 PM at the same position, using the average as the final result of each time measurement. The diurnal changes in P_n , stomatal conductance (g_s), intercellular CO₂ concentration (C_i), transpiration rate (T_r), photosynthetic photon flux density (PPFD), air temperature (T_a), air relative humidity (RH), and ambient CO₂ concentration (C_a) were measured simultaneously using the same method, and the data were recorded when the rate of CO₂ uptake had stabilized.

Dynamic changes of chlorophyll fluorescence parameters. All chlorophyll fluorescence parameters, including the maximal efficiency of PSII photochemistry (F_v/F_m), the effective efficiency of PSII photochemistry (F_v'/F_m'), actual PSII efficiency (Φ_{PSII}), photochemical quenching (qP), and nonphotochemical quenching (NPQ), were measured using a chlorophyll fluorescence imager (CF Imager; Techlogica, Essex, UK). All fluorescence measurements were performed five times following the first NPs treatment, once every 10 d. The leaves with consistent and good growth were selected, with six repeats per treatment. The plants were placed in the dark 30 min before measurement to obtain the dark fluorescence parameters. Following this, the actinic light of the illumination incubator was activated, and fluorescence was examined after adapting to the light of 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 5 min to obtain light fluorescence parameters.

Polysaccharide and moisture content. Polysaccharide content was determined according to the method described by Chen and Huang (2019). Root powder was weighed 10 g and placed in a 100-ml volumetric bottle, and 80 ml of 80% (v/v) ethanol was added and refluxed at 90°C for five times. After the precipitation was dissolved in water (80 ml), it was placed in a 90°C water bath for 1 h. The extract was filtered while it was hot, and then the precipitation was washed with distilled water several times; the washing liquid was incorporated into the filtrate, and after cooling, it was transferred to a 100-ml volumetric bottle. Finally, distilled water was used to make a constant volume for use. Each treatment was repeated three times. Under

Table 1. Effects of Fe₃O₄ NPs on growth and yield in *P. heterophylla* ($\bar{x} \pm \text{SD}$).^z

Treatment ^y	Relative growth yield		Root tuber			Yield Fresh wt (t·ha ⁻¹)
	Ht (%)	Diam (%)	Length (cm)	Diam (mm)	Dry wt (g/root)	
CK	43.78 ± 3.19 b	31.43 ± 2.56 bc	6.272 ± 0.541 b	5.439 ± 0.43 bc	0.351 ± 0.013 c	2.19 ± 0.11 c
Fe20	52.54 ± 3.60 a	34.43 ± 3.01 ab	7.823 ± 0.557 a	6.424 ± 0.41 ab	0.401 ± 0.009 b	2.59 ± 0.13 b
Fe50	59.75 ± 4.41 a	42.11 ± 3.38 a	8.637 ± 0.659 a	6.724 ± 0.52 a	0.441 ± 0.011 a	3.38 ± 0.15 a
Fe100	34.18 ± 3.07 c	26.56 ± 2.81 c	4.812 ± 0.402 c	5.012 ± 0.48 c	0.312 ± 0.014 d	1.82 ± 0.10 d

^zLowercase letters indicate $P < 0.05$ levels.^yCK = 0 mg·L⁻¹ Fe₃O₄ NPs; Fe20 = 20 mg·L⁻¹ Fe₃O₄ NPs; Fe50 = 50 mg·L⁻¹ Fe₃O₄ NPs; Fe100 = 100 mg·L⁻¹ Fe₃O₄ NPs.

the previously mentioned experimental conditions, polysaccharides were extracted, and absorbance was determined at 490 nm by a phenol-sulfuric acid method with a spectrophotometer. The moisture content was measured by the drying method.

Gray relational analysis (GRA). In this article, the GRA took P_n as the reference sequence and the factors that affect P_n as the comparative sequence. The original data were standardized by the initial value method, and the resolution coefficient was determined to 0.5. For the specific calculation method, refer to Xu et al. (2017).

Statistical analysis. Statistical analysis was performed using SPSS software (Version 22.0; IBM Corp., Armonk, NY). One-way analysis of variance and Tukey's multiple comparisons were used to determine the differences among treatments ($P < 0.05$). Pearson's correlation was used to investigate the relationships between the variables. Figures were prepared using OriginPro (Version 8.0; OriginLab Corp., Northampton, MA). Each treatment was conducted with three replicates, and the results were presented as mean ± SD.

Results

Effect of Fe₃O₄ NPs on growth and yield in *P. heterophylla*. As shown in Table 1, the relative growth increments of seedling height and ground diameter, the length, diameter, dry weight of the root tuber, and root tuber yield per unit area increased initially, and then these decreased with the concurrent increase in Fe₃O₄ NPs concentration in a dose-dependent manner. A concentration of 50 mg·L⁻¹ Fe₃O₄ NPs was found to optimally promote these indexes, which increased by 36.47%, 33.98%, 37.70%, 23.63%, 25.64%, and 54.33% ($P < 0.05$) compared with the control group. However, the treatment with the highest concentration of Fe₃O₄ NPs (100 mg·L⁻¹) resulted in a negative response. These results indicated that appropriate concentrations (20–50 mg·L⁻¹) of Fe₃O₄ NPs effectively promoted the growth and development of seedling, the accumulation of dry matter, the expansion of the root tuber, and yield of per unit area of *P. heterophylla*.

Effect of Fe₃O₄ NPs on dry matter distribution in *P. heterophylla*. As shown in Fig. 1, appropriate concentrations (20–50 mg·L⁻¹) of

Fe₃O₄ NPs not only significantly improved the biomass and root weight of *P. heterophylla*, but also significantly increased the distribution of dry matter in root. During the experiment, the root-to-shoot ratio (R/T) of each treatment group showed an overall increasing trend, and it increased significantly in the later stage; this change indicated that *P. heterophylla* growth was concentrated in the aboveground part during the early growth stage and gradually transferred to the underground part in the later growth period (Fig. 1B). The promoting effect of Fe₃O₄ NPs on R/T also showed an obvious concentration effect, and the effect of 50 mg·L⁻¹ treatment was the most significant—at 44.62% higher than the control group at 50 d (Fig. 1B). The distribution ratio of dry matter in the roots also showed the same result (Fig. 1C). Hence, Fe₃O₄ NPs might be promoting the transport and distribution of organic matter from aboveground to underground during root expansion of *P. heterophylla*. The concentration effect of all treatments could be ranked from greatest to least in order as follows: Fe50, Fe20, CK, and Fe100.

Effect of Fe₃O₄ NPs on chlorophyll content in *P. heterophylla* leaves. As shown in Fig. 2, during the experiment, SPAD values in all treatment groups increased at first and then decreased, reaching the peak at 30 days. The treatment with the ideal concentration (20–50 mg·L⁻¹) of Fe₃O₄ NPs had significantly higher SPAD values than those of the control group during the whole experiment. The effect of 50 mg·L⁻¹ treatment was the greatest among all treatments. High concentration (100 mg·L⁻¹) treatment had an inhibitory effect, and the resulting SPAD values were significantly lower than those of CK ($P < 0.05$) after 30 d of treatment, with an obvious concentration effect. On day 30 of the Fe₃O₄ NPs (20–50 mg·L⁻¹) treatment, the photosynthetic pigments (chlorophyll a and b) per plant were significantly increased compared with the control (Table 2). Priming with Fe₃O₄ NPs at 50 mg·L⁻¹ significantly decreased the ratio of chlorophyll a to b compared with the control (Table 2). These results revealed that appropriate concentrations of Fe₃O₄ NPs could effectively improve chlorophyll content.

Effect of Fe₃O₄ NPs on the net photosynthetic rate in *P. heterophylla* leaves. As shown in Fig. 3, during the experiment, the values of the P_n for all treatments increased at first, then decreased, and reached a peak at 30 d of treatment. The change trend of P_n was the same trend as that of chlorophyll content. The effect of

Fig. 1. Effect of Fe₃O₄ NPs on dry matter distribution in *P. heterophylla*. (A) Root weight. (B) R/T. (C) Dry matter distribution. (D) Biomass. R/T = root-to-shoot ratio; CK = 0 mg·L⁻¹ Fe₃O₄ NPs; Fe20 = 20 mg·L⁻¹ Fe₃O₄ NPs; Fe50 = 50 mg·L⁻¹ Fe₃O₄ NPs; Fe100 = 100 mg·L⁻¹ Fe₃O₄ NPs. Lowercase letters indicate $P < 0.05$ levels.

Fig. 2. Effect of Fe_3O_4 NPs on chlorophyll content in *P. heterophylla* leaves. SPAD = Soil and Plant Analyzer Development; CK = 0 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe20 = 20 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe50 = 50 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe100 = 100 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs. Lowercase letters indicate $P < 0.05$ levels.

the 50 $\text{mg}\cdot\text{L}^{-1}$ treatment was the greatest among all treatments. On day 30 of the treatment, P_n significantly increased by 30.11%. These results indicated that appropriate concentrations of Fe_3O_4 NPs could effectively increase chlorophyll content, thereby promoting photosynthesis.

Effects of Fe_3O_4 NPs on diurnal photosynthetic variation in *P. heterophylla* leaves. As shown in Fig. 4A, the diurnal variation of PPFD showed a single peak at 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The peak appeared at about 12:00 PM, indicating that the weather was clear on that day. The C_a values fluctuated moderately, but these maintained an average state of 400 $\mu\text{mol}\cdot\text{mol}^{-1}$. T_a also showed a single-peak diurnal variation, with a peak at 2:00 PM, which was 34 °C. RH showed an inverted parabola. A valley value appeared at 2:00 PM, followed by a gradual increase along with the decrease of PPFD and T_a (Fig. 4B).

As shown in Fig. 5A, all *P. heterophylla* leaf treatments triggered diurnal fluctuations in P_n and presented a double peak configuration: a discernible photosynthetic midday depression (11:00 AM to 1:00 PM) together with two peaks occurring at 10:00 AM and 2:00 PM. Compared with the control, the treatment with the ideal concentration of Fe_3O_4 NPs (20–50 $\text{mg}\cdot\text{L}^{-1}$) did not change the bimodal trend, but it significantly increased the daily mean and peak value of P_n and alleviated the phenomenon of “lunch break” (Fig. 5A). The effect of 50 $\text{mg}\cdot\text{L}^{-1}$ treatment was the best; and the daily mean, 10:00 AM peak, and 12:00 PM valley values increased by 40.13%, 40.24%, and 32.95%, respectively ($P < 0.05$) (Fig. 5A). However, the treatment with an

excessively high concentration (100 $\text{mg}\cdot\text{L}^{-1}$) had an inhibitory effect (Fig. 5A).

As shown in Fig. 5B and D, the changing trend of g_s and T_r was generally similar to P_n ; and there is also shown a bimodal trend. Treatment with an appropriate concentration of Fe_3O_4 NPs (20–50 $\text{mg}\cdot\text{L}^{-1}$) significantly promoted g_s and T_r . The effect of 50 $\text{mg}\cdot\text{L}^{-1}$ treatment was the strongest, while higher concentrations had a negative effect. Compared with P_n , the alleviating effect of Fe_3O_4 NPs on the “lunch break” phenomenon of g_s and T_r was more obvious. Contrary to the trend observed in g_s , the changing trend of C_i proceeded in the opposite direction to that of P_n (Fig. 5C). C_i in all *P. heterophylla* leaf treatments showed a W shape, with trough values at 10:00 AM and 2:00 PM, and peaks at 8:00 AM and 12:00 PM (Fig. 5C). Treatment with appropriate concentrations of Fe_3O_4 NPs (20–50 $\text{mg}\cdot\text{L}^{-1}$) significantly decreased C_i during diurnal variation, including daily mean value, peak value, and valley value. The effect of the 50 $\text{mg}\cdot\text{L}^{-1}$ treatment was the strongest, decreasing daily mean value, peak value, and valley value by 47.06%, 19.38%, and 67.03%, respectively ($P < 0.05$), compared with the control (Fig. 5C). The decrease in photosynthesis observed in *P. heterophylla* at noon could be attributed to nonstomatal limitation factors, given the regularity of the daily variations in P_n , g_s , and C_i .

Effects of Fe_3O_4 NPs on chlorophyll fluorescence parameters in *P. heterophylla* leaves. As shown in Fig. 6A–D, the values of F_v/F_m , F_v'/F_m' , Φ_{PSII} , and qP of all treatments increased at first and then decreased during the experiment. The above indexes increased initially, followed by a decrease in a dose-dependent manner associated with increasing concentrations of Fe_3O_4 NPs. Compared with the control, 20–50 $\text{mg}\cdot\text{L}^{-1}$ treatment effectively promoted the increase of the above indexes, and the promoting effect of 50 $\text{mg}\cdot\text{L}^{-1}$ treatment was the best. However, 100 $\text{mg}\cdot\text{L}^{-1}$ treatment had an inhibitory effect.

A contrary trend to other fluorescence parameters was observed in NPQ (Fig. 6E). All the treatment groups showed a downward trend at first and then an upward trend, reaching the valley value at 30 d of treatment. Appropriate concentrations of Fe_3O_4 NPs (20–50 $\text{mg}\cdot\text{L}^{-1}$) were shown to decrease NPQ compared with CK; but the highest concentration (100 $\text{mg}\cdot\text{L}^{-1}$) prompted the increase of NPQ. The treatment effect of 50 $\text{mg}\cdot\text{L}^{-1}$ was the strongest, which was 27.81% lower

Fig. 3. Effects of Fe_3O_4 NPs on the net photosynthetic rate (P_n) in *P. heterophylla* leaves. CK = 0 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe20 = 20 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe50 = 50 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe100 = 100 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs. Lowercase letters indicate $P < 0.05$ levels.

than that of CK on the 30th day ($P < 0.05$). The above results showed that the appropriate concentration of Fe_3O_4 NPs could effectively improve the PSII photochemical efficiency of *P. heterophylla* and reduce the energy dissipation of nonphotochemical regulation.

Effect of Fe_3O_4 NPs on polysaccharide and moisture content in *P. heterophylla* root tubers. As shown in Table 3, Application of Fe_3O_4 NPs at 50 $\text{mg}\cdot\text{L}^{-1}$ had a marked effect on polysaccharide content compared with the control. However, the treatment with the highest concentration of Fe_3O_4 NPs (100 $\text{mg}\cdot\text{L}^{-1}$) resulted in a negative response. There was almost no significant difference in water content among the treatments. These results might be due to the increased chlorophyll content from the Fe_3O_4 NPs foliar spray. In the case of foliar spray, this would result in a high photosynthesis rate and consequently more production of carbohydrate precursors for polysaccharide content.

Gray relational analysis. To further explore the influence of internal and external environmental factors on photosynthesis of *P. heterophylla* and the regulation of Fe_3O_4 NPs, as shown in Table 4, the correlation between P_n and physiological factors under all treatments was in the following order, from greatest to least: T_r , g_s , and C_i . The order of the gray correlation degree between P_n and environmental factors from greatest to least was as follows: T_a , C_a , PPFD, and RH. Fe_3O_4 NPs treatment of 50 $\text{mg}\cdot\text{L}^{-1}$ effectively reduced the correlation between net photosynthetic rate of *P. heterophylla* leaves and external environmental and physiological factors. Therefore, application of the appropriate mass concentration of Fe_3O_4 NPs treatment could reduce the impact of internal and external environmental factors on P_n of *P. heterophylla*.

Correlation analysis of the different indexes. As shown in Table 5, P_n was positively correlated with yield, seedling height, ground diameter, chlorophyll content, g_s , T_r , F_v/F_m , F_v'/F_m' , Φ_{PSII} , and qP, and negatively correlated with C_i and NPQ; and the correlation was significant ($P < 0.05$). Furthermore, chlorophyll content was positively correlated with P_n , F_v/F_m , and Φ_{PSII} ($P < 0.05$).

Table 2. Effects of Fe_3O_4 NPs on chlorophyll a and b content in *P. heterophylla* leaves ($\bar{x} \pm \text{sd}$).^z

Treatment ^y	Total Chl ($\text{mg}\cdot\text{g}^{-1}$)	Chla ($\text{mg}\cdot\text{g}^{-1}$)	Chlb ($\text{mg}\cdot\text{g}^{-1}$)	Chla/Chlb
CK	1.81 ± 0.05 c	1.36 ± 0.06 c	0.44 ± 0.07 bc	3.09 ± 0.06 a
Fe20	2.17 ± 0.11 b	1.62 ± 0.04 b	0.54 ± 0.03 b	3.00 ± 0.03 a
Fe50	2.55 ± 0.08 a	1.89 ± 0.12 a	0.65 ± 0.05 a	2.90 ± 0.04 b
Fe100	1.72 ± 0.07 cd	1.28 ± 0.15 cd	0.42 ± 0.03 c	3.05 ± 0.05 a

^zLowercase letters indicate $P < 0.05$ levels.

^yChl = chlorophyll; CK = 0 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe20 = 20 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe50 = 50 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe100 = 100 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs.

Fig. 4. Diurnal changes of (A) photosynthetic photon flux density (PPFD) and ambient CO₂ concentration (C_a), and (B) air relative humidity (RH) and air temperature (T_a). CK = 0 mg·L⁻¹ Fe₃O₄ NPs; Fe20 = 20 mg·L⁻¹ Fe₃O₄ NPs; Fe50 = 50 mg·L⁻¹ Fe₃O₄ NPs; Fe100 = 100 mg·L⁻¹ Fe₃O₄ NPs. Lowercase letters indicate *P* < 0.05 levels.

Discussion

Fe₃O₄ NPs have been frequently used in biomedical applications due to their magnetic properties (Ali et al., 2016). At present, few works have been done on evaluating their potential application in agriculture (Pradhan et al., 2019). Before the possible development of nano Fe fertilizers, there were interactions between iron oxide NPs and plants, and these must be understood. Our results indicated that foliar spraying with appropriate concentrations of Fe₃O₄ NPs (20–50 mg·L⁻¹) could significantly promote the photosynthesis of *P. heterophylla*, thereby promoting growth and increasing root yield per unit area under field conditions. Plaksenkova et al. (2019) also found that Fe₃O₄ NPs (1–2 mg·L⁻¹) had a positive effect on the growth and development of rocket (*Eruca sativa*). At the same time, Fe₃O₄ NPs dramatically increased the distribution of dry matter in root and polysaccharide content of root, which showed that Fe₃O₄ NPs promoted the synthesis of organic matters. This further proved that the introduction of iron oxide nanoparticles into plants could promote the distribution and accumulation of organic matter to underground parts to a certain extent and indirectly promote the growth of root tubers. Konate et al. (2018) found that Fe₃O₄ NPs could interact with plants to produce OH⁻ free radicals, which could stimulate the degradation of pectin in the plant cell wall and relax the root cell wall to promote plant root growth. Like the study by Chen et al. (2018), our results indicated that different Fe₃O₄ NPs concentrations influenced plant morphology. And at high concentrations, Fe₃O₄ NPs were found to inhibit growth. When Fe₃O₄ NPs accumulate

excessively in plants, they might accumulate in some cells, leading to stress effects (Bystrzejska-Piotrowska et al., 2012). However, Giordani et al. (2012) noted that Fe₃O₄ NPs did not cause any harmful effects to tomato (*Solanum lycopersicum*) plants. The controversy in reported results could be mainly attributed to the difference of size in nanomaterials, plant species, or culture condition used in different studies. Consequently, more studies are required to fully understand the effects of Fe-based nanomaterials before they can be applied safely in agriculture.

Chloroplast in plant leaves is highly sensitive to iron oxide NPs (Li et al., 2018). Our results revealed that the chlorophyll content increased significantly. Fe₃O₄ NPs (6–10 nm in size) promoted the dry biomass and chlorophyll concentration of *Quercus macdougalii* (Pariona et al., 2017). One possible explanation was that Fe₃O₄ NPs promoted the reduction and use of iron by increasing the activity of iron oxygen reductase, thus indirectly promoting the metabolism of porphyrin to synthesize chlorophyll precursor, namely δ-aminolevulinic acid (Maswada et al., 2018). Chlorophyll is the main pigment in photosynthesis. Photosynthetic pigments are the basis for absorbing and transferring light energy (Ruiz-Espinoza et al., 2010). The content of chlorophyll (Chl) components and Chla/Chlb are important indicators to characterize the physiological status of plant photosynthetic tissues, which significantly affect plant photosynthesis (Feng et al., 2003). During the whole experiment, *P_n* was consistent with the change of SPAD. Fe₃O₄ NPs (20–50 mg·L⁻¹) had a positive effect on the photosynthetic pigments (Chla and Chlb) content. *P_n* are significantly correlated with

chlorophyll content, which suggests that Fe₃O₄ NPs can promote the enhancement of photosynthesis by increasing the chlorophyll content. Enhanced photosynthetic activity might be due to improving the size and yield of the chloroplast and the concentration of rubisco protein (Timperio et al., 2007). However, the specific mechanism needs further investigation. Yan et al. (2020) found that Fe₃O₄ NPs had no impact on maize leaf chlorophyll biosynthesis or photosynthetic activity. Differences in application position, growth environment, and plant species may explain these differences.

Photosynthesis is the only energy entry point in plants, so it also serves as a sensor to understand plant metabolism and physiology (Rastogi et al., 2017). Correlation analysis indicated that *P_n* was significantly positively correlated with yield, suggesting that the yield-increasing mechanism of Fe₃O₄ NPs nanoparticles in *P. heterophylla* was largely attributed to the enhancement of photosynthesis. In parallel to our findings, a previous study by Tombuloglu et al. (2019) reported that Fe₃O₄ NPs (0–13 nm in size) promoted the growth enhancement of barley, attributable to the increased level of pigmentation and the photosynthetic gene expressions. The reason why Fe₃O₄ NPs increase net photosynthesis remains unknown. Diurnal variations in photosynthesis reflect the substance accumulation and physiological metabolism sustainability of plants during the day and can also be used to explore the limited factors affecting photosynthesis by analyzing the variation in gas exchange parameters. Appropriate concentrations of Fe₃O₄ NPs (20–50 mg·L⁻¹) did not alter the diurnal fluctuations in the *P_n* double-peak pattern observed in *P. heterophylla* leaves. They did significantly improve *P_n* throughout the entire day and alleviated the “photosynthetic lunch break” phenomenon when compared with the control. Either condition may lead to an increase in the photosynthetic rate: one is the increase in stomatal opening (reduced resistance to the entry of CO₂), and thereby increased CO₂ uptake activity at the chloroplast level (Alidoust and Isoda, 2013). The other is the increase in enzymatic activity at the chloroplast level, which is manifested as a decrease in *C_i* value (Farquhar

Table 3. Effects of Fe₃O₄ NPs on polysaccharide and moisture content in *P. heterophylla* root tubers ($\bar{x} \pm \text{SD}$).^z

Treatment ^y	Moisture (%)	Polysaccharide (%)
CK	70.11 ± 0.67 a	17.43 ± 0.36 c
Fe20	70.48 ± 0.51 a	18.51 ± 0.31 b
Fe50	71.15 ± 0.74 a	20.02 ± 0.42 a
Fe100	68.58 ± 0.47 ab	16.16 ± 0.28 c

^zLowercase letters indicate *P* < 0.05 levels.

^yCK = 0 mg·L⁻¹ Fe₃O₄ NPs; Fe20 = 20 mg·L⁻¹ Fe₃O₄ NPs; Fe50 = 50 mg·L⁻¹ Fe₃O₄ NPs; Fe100 = 100 mg·L⁻¹ Fe₃O₄ NPs.

Fig. 5. Effects of Fe_3O_4 NPs on diurnal variations of (A) net photosynthetic rate (P_n), (B) stomatal conductance (g_s), (C) intercellular CO_2 concentrations (C_i), and (D) transpiration rate (T_r) in *P. heterophylla*. CK = 0 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe20 = 20 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe50 = 50 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe100 = 100 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs. Lowercase letters indicate $P < 0.05$ levels.

and Sharkey, 1982). The increase in g_s readings, along with the decrease in C_i values, strongly suggest that the changes in photosynthetic rate during the “noon break” in this study were mainly due to nonstomatal limiting factors. Specifically, Fe_3O_4 NPs enhanced the photosynthetic capacity of *P. heterophylla* by improving the photosynthetic activity of mesophyll cells

during the midday photosynthetic depression. In addition, the increase in P_n was accompanied by the increase in g_s during the entire diurnal change process, indicating that Fe_3O_4 NPs can also increase the photosynthetic performance by increasing the stomata opening.

Chlorophyll fluorescence analyses are often used to detect the response of plant

photosynthetic mechanisms to external factors and the mechanism of those responses (Li et al., 2020a). The results of the chlorophyll fluorescence analyses indicated that Fe_3O_4 NPs (20–50 $\text{mg}\cdot\text{L}^{-1}$) had a clear, positive influence on F_v/F_m , F_v'/F_m' , Φ_{PSII} , and qP compared with the control, suggesting that it effectively promoted the light energy absorption, transfer, and transformation efficiency of PSII. On the surface of Fe_3O_4 NPs, there are two valences of Fe (Fe^{2+} and Fe^{3+}), which might be act as both electron donor and acceptor (Jalali et al., 2016). Fe_3O_4 NPs improved the opening rate of the PSII reaction center and the activity of the electron transport chain under light conditions, thereby enhancing the photochemical reaction of mesophyll cells. The actual photochemical efficiency and quantum yield of PSII were the main factors that improved P_n . Maswada et al. (2018) found that iron oxide nanoparticles could improve the efficiency of electron transfer by increasing the absorption of photons from PSII antennas to Q_B , thus improving the efficiency of PSII light reactions. Furthermore, chlorophyll content was positively correlated with P_n , F_v/F_m , and Φ_{PSII} . Chla/Chlb decreased with Fe_3O_4 NPs (50 $\text{mg}\cdot\text{L}^{-1}$) treatment. Chla/Chlb characterizes the proportion of light-harvesting complex of PSII (LHC-II) in all chlorophyll-containing structures. This value was negatively correlated with LHC-II content (Feng et al., 2003), indicating that Fe_3O_4 NPs (50 $\text{mg}\cdot\text{L}^{-1}$) can promote the increase of LHC-II content and ensuring that plants can capture and transfer more light energy for photosynthesis. Li et al. (2020b) used DCPIP reduction assay and fluorescence microscopy observation to prove that Fe_3O_4 NPs binds to chloroplasts

Fig. 6. Effects of Fe_3O_4 NPs on (A) the maximal efficiency of PSII photochemistry (F_v/F_m), (B) the effective efficiency of PSII photochemistry (F_v'/F_m'), (C) actual PSII efficiency (Φ_{PSII}), (D) photochemical quenching (qP), and (E) nonphotochemistry quenching (NPQ) in *P. heterophylla* seedlings. CK = 0 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe20 = 20 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe50 = 50 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs; Fe100 = 100 $\text{mg}\cdot\text{L}^{-1}$ Fe_3O_4 NPs. Lowercase letters indicate $P < 0.05$ levels.

Table 4. Gray relational degree between net photosynthetic rate and physiological and ecological factors.

Reference sequence ^z	Compare the sequence ^y						
P_n	PPFD	C_a	RH	T_a	g_s	C_i	T_r
CK	0.8626	0.8640	0.8002	0.8665	0.7981	0.7695	0.8073
Fe50 (50 mg·L ⁻¹)	0.6657	0.6689	0.6634	0.6723	0.6714	0.6501	0.6717

^z P_n = net photosynthetic rate; CK = 0 mg·L⁻¹ Fe₃O₄ NPs; Fe50 = 50 mg·L⁻¹ Fe₃O₄ NPs.

^yPPFD = photosynthetic photon flux density; C_a = ambient CO₂ concentration; RH = atmospheric relative humidity; T_a = atmospheric temperature; g_s = stomatal conductance; C_i = intercellular CO₂; T_r = transpiration rate.

Table 5. The correlation analysis of different indexes.

Variable ^z	YPA	SH	GD	Chl	P_n	g_s	C_i	T_r	F_v/F_m	F_v'/F_m'	Φ_{PSII}	qP	NPQ
YPA	1												
SH	0.989**	1											
GD	0.876**	0.793**	1										
Chl	0.866**	0.829**	0.925**	1									
P_n	0.958**	0.916**	0.830**	0.918**	1								
g_s	0.882**	0.776**	0.851**	0.797**	0.610**	1							
C_i	-0.771**	-0.689**	-0.753**	-0.742**	-0.768**	-0.745**	1						
T_r	0.789**	0.668**	0.765**	0.755**	0.845**	0.672**	-0.862**	1					
F_v/F_m	0.992**	0.897**	0.927**	0.828**	0.794**	0.740**	-0.934**	0.829**	1				
F_v'/F_m'	0.897**	0.828**	0.815**	0.823**	0.667**	0.543**	-0.742**	0.642**	0.725**	1			
Φ_{PSII}	0.813**	0.798**	0.897**	0.841**	0.733**	0.562**	-0.759**	0.763**	0.765**	0.609**	1		
qP	0.888**	0.902**	0.910**	0.816**	0.780**	0.614**	-0.834**	0.781**	0.828**	0.888**	0.905**	1	
NPQ	-0.736**	-0.786**	-0.826**	-0.735**	-0.455*	-0.801**	0.726**	-0.509*	-0.727**	-0.727**	-0.559**	-0.711**	1

^zYPA = yield per area; SH = seeding height; GD = ground diameter; Chl = chlorophyll; P_n = net photosynthetic rate; g_s = stomatal conductance; C_i = intercellular CO₂ concentration; T_r = transpiration rate; F_v/F_m = the maximal efficiency of PSII photochemistry; F_v'/F_m' = the effective efficiency of PSII photochemistry; Φ_{PSII} = actual PSII efficiency; qP = photochemical quenching; NPQ = nonphotochemical quenching.

*Significant correlation at the 0.05 level (both sides); **significant correlation at the 0.01 level (both sides); n = 24.

instead of released Fe³⁺/Fe²⁺, and increases the photosynthesis by enhancing the PSII activities. The appropriate concentration of Fe₃O₄ NPs treatment could not only improve qP but also significantly could reduce NPQ. Our results showed that Fe₃O₄ NPs treatment could effectively alleviate the thermal dissipation caused by excessive excitation energy under strong light and high temperature, which might reflect the alleviation of the photoinhibition of *P. heterophylla*. This was achieved by enhancing the photochemical use of excess excitation energy.

As one of the main bioactive components of *P. heterophylla*, polysaccharides have antioxidant, immune, and antitumor physiological functions (Varghese et al., 2019). Polysaccharides have become one of the focuses of Chinese herbal medicine extraction and modernization research for many years due to their good biological activities and clinical effects (Chen and Huang, 2019). Fe₃O₄ NPs induced an increase in the polysaccharides of *P. heterophylla* compared with the control. Polysaccharides are the main components of root exudates, which are composed of photosynthetically fixed carbon and are secreted by plant roots (Cairney et al., 1989). This result confirmed that Fe₃O₄ NPs enhanced the secretion of organic matter in roots by promoting photosynthetic carbon assimilation.

Gray correlation analysis could be used to study the dynamic tendency of different factors and to explore the correlation degree of the consistency of dynamic trends between P_n and environmental factors. The gray correlation analysis further showed that the correlation between P_n and internal and external environmental factors affecting *P. heterophylla* was significantly lower than that of the control after the treatment of a

suitable concentration of Fe₃O₄ NPs. This showed that Fe₃O₄ NPs treatment could enhance the adaptability of *P. heterophylla* to changes in the internal and external environment. Indeed, alleviation of plant stress by nanomaterials has been reported recently—for example, the use of Fe₃O₄ NPs to reduce the oxidative stress of rice plants caused by iron deficiency (Li et al., 2021). However, to our knowledge, few studies have investigated whether foliar-applied Fe₃O₄ NPs could alleviate photoinhibition or not. Although high temperatures, strong light, and other environmental factors cause photoinhibition easily, our research has found that plants treated with appropriate concentrations of Fe₃O₄ NPs could still maintain a relatively stable P_n , showing strong environmental adaptability. This may also be related to environmental conditions or plant characteristics. Hence, our results would provide a new perspective for the application of Fe₃O₄ NPs as fertilizers in alleviating stress.

Conclusions

The present study showed that foliar spraying with appropriate concentrations of Fe₃O₄ NPs (20–50 mg·L⁻¹) could effectively promote the growth and development of root tubers and significantly increase the yield of *P. heterophylla*. Its yield-increasing effect was linked to improving photosynthetic performance, promoting the synthesis of organic matter, and aiding its distribution and accumulation in the root system. It is noteworthy that Fe₃O₄ NPs caused positive effects on polysaccharides content. The enhancement of the photochemical activity of mesophyll cells and the improvement of light energy use efficiency were the main mechanisms for improving photosynthetic performance

and adaptability to photo-inhibiting environments. Furthermore, Fe₃O₄ NPs can affect different aspects of plant growth, and the effects are dose dependent. In summary, our study helped us to better understand how Fe₃O₄ NPs impact medicinal plants from the aspects of the photosynthetic physiological levels, and our work provided useful information on their agricultural applications. However, when considering medicinal plants, the effect of iron oxide nanoparticles on molecular levels of *P. heterophylla* needs to be further discussed. Fe₃O₄ NPs might be an ideal substitution for the traditional fertilizer, although further studies are still needed to thoroughly assess its potential risk to the environmental and food security.

Literature Cited

- Alidoust, D. and A. Isoda. 2013. Effect of γ -Fe₂O₃ nanoparticles on photosynthetic characteristic of soybean (*Glycine max* [L.] Merr): Foliar spray versus soil amendment. *Acta Physiol. Plant.* 35:3365–3375, doi: 10.1007/s11738-013-1369-8.
- Ali, A., H. Zafa, M. Zia, U.H. Ihsan, P.A. Rahman, A.J. Sarfraz, and H. Altaf. 2016. Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. *Nanotechnol. Sci. Appl.* 9:49–67, doi: 10.2147/NSA.S99986.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24:1–16, doi: 10.1104/pp.24.1.1.
- Bystrzejewska-Piotrowska, G., M. Asztemborska, R. Steborowski, H. Polkowska-Motrenko, D. Bozena, and R. Justyna. 2012. Application of neutron activation for investigation of Fe₃O₄ nanoparticles accumulation by plants. *Nukleonika* 57:427–430, doi: 10.1524/ract.2012.1961.

- Cai, L., L.T. Cai, H.Y. Jia, C.Y. Liu, D.B. Wang, and X.C. Sun. 2020. Foliar exposure of Fe₃O₄ nanoparticles on *Nicotiana benthamiana*: Evidence for nanoparticles uptake, plant growth promoter and defense response elicitor against plant virus. *J. Hazard. Mater.* 393:122415, doi: 10.1016/j.jhazmat.2020.122415.
- Cairney, J.W.G., A.E. Ashfoed, and W.G. Allaway. 1989. Distribution of photosynthetically fixed carbon within root systems of *Eucalyptus pilularis* plants ectomycorrhizal with *Pisolithus tinctorius*. *New Phytol.* 112:495–500, doi: 10.1111/j.1469-8137.1989.tb00343.x.
- Chen, F. and G.L. Huang. 2019. Antioxidant activity of polysaccharides from different sources of ginseng. *Int. J. Biol. Macromol.* 125:906–908, doi: 10.1016/j.ijbiomac.2018.12.134.
- Chen, H.P., W.W. Zhang, X.P. Yang, P. Wang, S.P. McGrath, and F.J. Zhao. 2018. Effective methods to reduce cadmium accumulation in rice grain. *Chemosphere* 207:699–707, doi: 10.1016/j.chemosphere.2018.05.143.
- Chen, J.L., W.S. Pang, Y.J. Kan, L. Zhao, Z.D. He, W.T. Shi, B. Yan, H. Chen, and J. Hu. 2017. Structure of a pectic polysaccharide from *Pseudostellaria heterophylla* and stimulating insulin secretion of INS-1 cell and distributing in rats by oral. *Int. J. Biol. Macromol.* 2017: 456–463, doi: 10.1016/j.ijbiomac.2017.08.034.
- Chouliaras, V., I. Therios, A. Molassiotis, A. Patakas, and G. Diamantidis. 2004. Effect of iron deficiency on gas exchange and catalase and peroxidase activity in citrus. *J. Plant Nutr.* 27: 2085–2099, doi: 10.1081/PLN-200034638.
- Ding, Y.Y., X. Bai, Z.F. Ye, L.Y. Ma, and L. Liang. 2019. Toxicological responses of Fe₃O₄ nanoparticles on *Eichhornia crassipes* and associated plant transportation. *Sci. Total Environ.* 671: 558–567, doi: 10.1016/j.scitotenv.2019.03.344.
- Elfiky, S.A., M.A. Mohammed, M.S. Khater, Y.A.H. Osman, and E. Elsherbini. 2013. Effect of magnetite nano-fertilizer on growth and yield of *Ocimum basilicum* L. *Int. J. Indigenous Med. Plants* 463: 2051–4263, doi: 10.22067/jag.v2i1.7603.
- Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* 33:317–345, doi: 10.1146/annurev.pp.33.060182.001533.
- Feng, Y.J., B.S. Shi, G.M. Dong, and Q. Zhang. 2003. The application of chlorophyll fluorescence kinetics in plant stress and fruit storage. *J. Hebei Agr. Uni.* 26:89–92, doi: 10.3969/j.issn.1000–1573.2003.z1.026.
- Ghafariyan, M., M. Malakouti, M. Dadpour, P. Stroeve, and M. Mahmoudi. 2013. Effects of magnetite nanoparticles on soybean chlorophyll. *Environ. Sci. Technol.* 47:10645–10652, doi: 10.1021/es402249b.
- Giordani, T., A. Fabrizi, L. Guidi, L. Natali, G. Giunti, F. Ravasi, A. Cavallini, and A. Pardossi. 2012. Response of tomato plants exposed to treatment with nanoparticles. *EQA - Intl. J. Environ. Quality* 8: 27–38, doi: 10.6092/issn.2281-4485/3748.
- Gonzalez-Melendi, P., R. Fernandez-Pacheco, M.J. Coronado, E. Corredor, P.S. Testillano, M.C. Risueno, C. Marquina, M.R. Ibarra, D. Rubiales, and A. Perezde-Luque. 2008. Nanoparticles as smart treatment-delivery systems in plants: Assessment of different techniques of microscopy for their visualization in plant tissues. *Ann. Bot.* 10:1187–1195, doi: 10.1093/aob/mcm283.
- Gu, J.L. 2019. Characteristics and cultivation techniques of *Radix Pseudostellariae*. *Modern Agr. Sci. Technol.* (in Chin.) 6:42–44, doi: Kns.cnki.net/kcms/detail/detail.aspx.
- Herrmann, H.A., J. Schwartz, and G.N. Johnson. 2019. Metabolic acclimation: A key to enhancing photosynthesis in changing environments? *J. Expt. Bot.* 70:3043–3056, doi: 10.1093/jxb/erz157.
- Jalali, M., F. Chanarti, and A.M. Modarres-Sanavi. 2016. Effect of Fe₃O₄ nanoparticles and iron chelate on the antioxidant capacity and nutritional value of soil-cultivated maize (*Zea mays*) plants. *Crop Pasture Sci.* 67:621–628, doi: 10.1071/CP15271.
- Konate, A., Y.Y. Wang, X. He, M. Adeel, P. Zhang, Y.H. Ma, Y.Y. Ding, J.Z. Zhang, J. Yang, S. Kiztio, Y.K. Rui, and Z.Y. Zhang. 2018. Comparative effects of nano and bulk-Fe₃O₄ on the growth of cucumber (*Cucumis sativus*). *Ecotoxicol. Environ. Saf.* 165:547–554, doi: 10.1016/j.ecoenv.2018.09.053.
- Li, J., Y.G. Zhang, L.H. Gu, Z.H. Li, J. Li, Q. Zhang, Z.Y. Zhang, and L. Song. 2020a. Seasonal variations in the relationship between sun-induced chlorophyll fluorescence and photosynthetic capacity from the leaf to canopy level in a rice crop. *J. Expt. Bot.* 71(22):7179–7197, doi: 10.1093/jxb/eraa408.
- Li, J., W. Zhen, D. Long, L. Ding, A. Gong, C. Xiao, W. Jiang, X. Liu, T. Zhou, and L. Huang. 2016. De novo sequencing and assembly analysis of *Pseudostellaria heterophylla* transcriptome. *PLoS One* 11:e0164235, doi: 10.1371/journal.pone.0170134.
- Li, J.L., J. Hu, L. Xiao, Y.Q. Wang, and X.L. Wang. 2018. Interaction mechanisms between α -Fe₂O₃, γ -Fe₂O₃ and Fe₃O₄ nanoparticles and *Citrus maxima* seedlings. *Sci. Total Environ.* 625:677–685, doi: 10.1371/journal.pone.0170134.
- Li, M.S., P. Zhang, M. Adeel, Z.L. Guo, A.J. Chatwynd, C.N. Ma, T.G. Bai, Y. Hao, and Y.K. Rui. 2021. Physiological impacts of zero valent iron, Fe₃O₄ and Fe₂O₃ nanoparticles in rice plants and their potential as Fe fertilizers. *Environ. Pollut.* 269:116134, doi: 10.1016/j.envpol.2020.116134.
- Li, P.Y., A.D. Wang, W.C. Du, L. Mao, Z.B. Wei, S.F. Wang, H.Y. Yuan, R. Ji, and L.J. Zhao. 2020b. Insight into the interaction between Fe-based nanomaterials and maize (*Zea mays*) plants at metabolic level. *Sci. Total Environ.* 738:139795, doi: 10.1016/j.scitotenv.2020.139795.
- Liu, X.X. and J.S. Ruan. 2017. Protective effects of *Radix Pseudostellariae* polysaccharides on myocardial ischemia in rats. *Chinese J. Ethnomedicine & Ethnopharmacology* 26:18–20, doi: Cnki:sun:mzmj.0.2017-17-007.
- Ma, Y.L., H. Gu, Y. Zhang, L. Da, J. Li, F. Jang, D. Xia, and Y.F. Xie. 2017a. Effects of cerium nitrate on growth and photosynthetic characteristics of *Pseudostellaria heterophylla*. *J. Nanjing Forestry University (Natural Sci. Edition)* 41:71–79, doi: 10.3969/j.issn.1000–2006.2016.08009.
- Ma, Y.L., H.C. Zou, H. Gu, D.W. Shi, W.X. Gao, and Y.F. Zhang. 2017b. Stimulatory effect of lanthanum nitrate on the root tuber yield of *Pseudostellaria heterophylla* via improved photosynthetic characteristics. *J. Rare Earths* 35: 610–620, doi: 10.1016/S1002-0721(17)60954-2.
- Mahboobeh, J.L., F. Ghanati, and A.M. Modarres-Sanavi. 2016. Effect of Fe₃O₄ nanoparticles and iron chelate on the antioxidant capacity and nutritional value of soil-cultivated maize (*Zea mays*) plants. *Crop Pasture Sci.* 67:621–628, doi: 10.1071/CP15271.
- Maswada, H.F., M. Djanaguiraman, and P.V.V. Prasad. 2018. Seed treatment with nano-iron (III) oxide enhances germination, seedling growth and salinity tolerance of sorghum. *J. Agron. Crop Sci.* 204:577–587, doi: 10.1111/jac.1228010.
- Pariona, N., A.I. Martínez, H. Hernandez-Flores, and R. Clark-Tapia. 2017. Effect of magnetite nanoparticles on the germination and early growth of *Quercus macdougalii*. *Sci. Total Environ.* 575:869–875, doi: 10.1016/j.scitotenv.2016.09.128.
- Parisi, C., M. Vigani, and E. Rodríguez-Cerezo. 2015. Agricultural nanotechnologies: What are the current possibilities? *Nan. Today* 10:124–127, doi: 10.1016/j.nantod.2014.09.009.
- Plaksenkova, I., M. JermaJonoka, L. Bankovska, I. Gavarane, V. Gerbreder, E. Sledevskis, J. Snikeris, and I. Kokina. 2019. Effects of Fe₃O₄ nanoparticle stress on the growth and development of rocket *Eruca sativa*. *J. Nanomater.* 2019:1–10, doi: 10.1155/2019/2678247.
- Pradhan, S., S. Barik, and A. Goswami. 2019. Assessment of photo-modulation, nutrient-use efficiency and toxicity of iron nanoparticles in *Vigna radiata*. *Environ. Sci. Nano* 6:2544–2552, doi: 10.1039/C9EN00559E.
- Qiu, N.W., Z.Q. Ma, F.D. Wang, and X.A. Wang. 2007. A method for measuring plant relative growth yield. *Shandong Sci.* 20:24–28, doi: 10.3969/j.issn.1002-4026.2007.02.006.
- Raliya, R., V. Saharan, C. Dimkpa, and P. Biswas. 2018. Nanofertilizer for precision and sustainable agriculture: Current state and future perspectives. *J. Agr. Food Chem.* 2017:acs.jafc.7b02178, doi: 10.1021/acs.jafc.7b02178.
- Rastogi, A., M. Zivcak, O. Sytar, H.M. Kalaji, X. He, S. Mbarki, and M. Brestic. 2017. Impact of metal and metal oxide nanoparticles on plant: A critical review. *Front Chem.* 5:78, doi: 10.3389/fchem.2017.0078.
- Ruiz-Espinoza, F.H., B. Murillo-Amador, J.L. García-Hernández, L. Fenech-Larios, E.O. Rueda-Puente, E. Troyo-Díez, C. Kaya, and A. Beltrán-Morales. 2010. Field evaluation of the relationship between chlorophyll content in basil leaves and a portable chlorophyll meter (SPAD-502) readings. *J. Plant Nutr.* 33: 423–438, doi: 10.1080/01904160903470463.
- Rui, M., C. Ma, H. Yi, G. Jing, Y. Rui, X. Tang, Z. Qi, F. Xing, Z. Zhang, T. Hou, and S. Zhu. 2016. Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). *Front. Plant Sci.* 7:815, doi: 10.3389/fpls.2016.00815.
- Sheng, R., X.X. Xu, Q. Tang, D.F. Bian, Y. Li, C. Qian, X. He, X.H. Gao, R. Pan, and C. Wang. 2009. Polysaccharide of *Radix Pseudostellariae* improves chronic fatigue syndrome induced by poly in mice. *Evid. Based Complement. Alternat. Med.* 12:840516, doi: 10.1093/ecam/nep208.
- Sun, H.W., K.Q. Shi, K. Qi, H.Y. Kong, and M.T. Zou. 2020. *Pseudostellaria heterophylla* extract polysaccharide H-1-2 suppresses pancreatic cancer by inhibiting hypoxia-induced anterior gradient 2. *Mol. Ther. Oncolytics* 17: doi: 10.1016/j.omto.2020.03.007.
- Timperio, A.M., G.M. Amici, C. Barta, F. Loreto, and L. Zolla. 2007. Proteomics, pigment composition, and organization of thylakoid membranes in iron-deficient spinach leaves. *J. Expt. Bot.* 58:3695–3710, doi: 10.1093/jxb/erm219.
- Tombuloglu, H., Y. Slimani, G. Tombuloglu, M. Almessiere, and A. Baykal. 2019. Uptake and translocation of magnetite (Fe₃O₄) nanoparticles and its impact on photosynthetic genes in barley (*Hordeum vulgare* [L.]). *Chemosphere* 226:110–122, doi: 10.1016/j.chemosphere.2019.03.075.
- Tripathi, D.K., S. Shweta, S. Singh, S. Singh, R. Pandey, V.P. Singh, N.C. Sharma, S.M. Prasad, N.K. Dubey, and D.K. Chauhan. 2017. An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and

- phytotoxicity. *Plant Physiol. Biochem.* 110:2–12, doi: 10.1016/j.plaphy.2016.07.030.
- Varghese, S., M.M. Joseph, S.R. Aravind, B.S. Unnikrishnan, K.R. Pillai, and T.T. Sreelekha. 2019. Immunostimulatory plant polysaccharides impede cancer progression and metastasis by avoiding off-target effects. *Intl. Immunopharmacol.* 73:280–292, doi: 10.1016/j.intimp.2019.05.025.
- Wang, H., X. Kou, Z. Pei, J.Q. Xiao, X. Shan, and B. Xing. 2011. Physiological effects of magnetite (Fe₃O₄) nanoparticles on perennial ryegrass (*Lolium perenne* [L.]) and pumpkin (*Cucurbita mixta*) plants. *Nanotoxicology* 5:30e42, doi: 10.3109/17435390.2010.489206.
- Wang, P., E. Lombi, F.J. Zhao, and P.M. Kopittke. 2016. Nanotechnology: A new opportunity in plant sciences. *Trends Plant Sci.* 21:699e712, doi: 10.1016/j.tplants.2016.04.005.
- Wang, Y.Q., S.X. Wang, M.X. Xu, L. Xiao, Z.Y. Dai, and J.L. Li. 2019. The impacts of γ -Fe₂O₃ and Fe₃O₄ nanoparticles on the physiology and fruit quality of muskmelon (*Cucumis melo*) plants. *Environ. Pollut.* 249:1011–1018, doi: 10.1016/j.envpol.2019.03.119.
- Xu, M., Q. Zhu, J. Wu, Y. He, G. Yang, X. Zhang, L. Li, X. Yu, H. Peng, and L. Wang. 2017. Gray relational analysis for evaluating the effects of different rates of wine lees-derived biochar application on a plant-soil system with multi-metal contamination. *Environ. Sci. Pollut. R.* 25:6990–7001, doi: 10.1007/s11356-017-1048-1.
- Xu, X.X., Y.X. Huang, L.Z. Xia, and R. Zhang. 2012. The effect of *Pseudostellaria heterophylla* polysaccharides on antioxidant capacity and pancreatic pathology in diabetic mice. *Food Ind. Sci. Technol.* 33:392–393, doi: 10.13386/j.issn1002-0306.2012.24.007.
- Yan, L., P.Y. Li, X.P. Zhao, R. Ji, and L.J. Zhao. 2020. Physiological and metabolic responses of maize (*Zea mays*) plants to Fe₃O₄ nanoparticles. *Sci. Total Environ.* 718:137400, doi: 10.1016/j.scitotenv.2020.137400.
- Zhang, Y.D. 2020. Reevaluation on antioxidant activity of tonic chinese herbs *Pseudostellaria heterophylla*. *Animal Husbandry & Feed Sci.* 2020:11–16, doi: 10.19578/j.cnki.ahfs.2020.01.003.
- Zhao, Y.P., S. Lin, L. Chu, J.T. Gao, S. Azeem, and W. Lin. 2016. Insight into structure dynamics of soil microbiota mediated by the richness of replanted *Pseudostellaria heterophylla*. *Sci. Rep. UK* 6:26175, doi: 10.1038/srep26175.
- Zhu, H., J. Han, J.Q. Xiao, and Y. Jin. 2008. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *J. Environ. Monit.* 10:713e717, doi: 10.1039/b805998e.
- Zuverza-Mena, N., D. Martinez-Fernandez, W. Du, J.A. Hernandez-Viezcas, N. Bonilla-Bird, M.L. Lopez-Moreno, M. Komarek, J.R. Peralta-Videa, and J.L. Gardea-Torresdey. 2017. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses—A review. *Plant Physiol. Biochem.* 110:236–264, doi: 10.1016/j.plaphy.2016.05.03.