

Effects of nitrogen, phosphorus and potassium fertilization on photosynthetic characteristics, yield and quality of *Pulsatilla chinensis*

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Abstract: The effects of nitrogen, phosphorus and potassium fertilization on photosynthetic characteristics, yield and quality of *Pulsatilla chinensis* were studied in order to provide theoretical basis for standardized production of *Pulsatilla chinensis*. Field experiments were conducted with three factors quadratic saturation and D- optimal design, and photosynthetic parameters, dry matter quality and total saponin content of leaves were measured. The results showed that *Pulsatilla chinensis* had the greatest demand for nitrogen fertilizer, phosphate fertilizer had greater influence on photosynthetic characteristics, root growth and quality, and potassium fertilizer had more obvious influence on the growth of aboveground parts. In field cultivation, *Pulsatilla* should be properly proportioned with comprehensive consideration of yield and quality. the optimal fertilization scheme is nitrogen 180 kg / hm², phosphorus 225 kg / hm² and potassium 79.8 kg / hm².

Keywords: *Pulsatilla chinensis*; Photosynthetic characteristics; Output; Total saponin content

1. INTRODUCTION

Pulsatilla chinensis Regel is a perennial herb of *Pulsatilla* in Ranunculaceae, native to China, distributed in northeast China, north China, east China and other regions, like light, and born in wasteland and wilderness^[1]. Roots, leaves and flowers of *Pulsatilla chinensis* can be used as medicines, which belongs to traditional Chinese medicinal materials in China and is mainly effective as saponin (*Pulsatilla* saponin A₃, *Pulsatilla* saponin III, cephalin B₄). pharmacological studies show that it has antitumor, anti-parasitic and antioxidant effects^[2]. At present, the research on *Pulsatilla chinensis* mostly focuses on chemical composition^[3], pharmacological action^[4], tissue culture and propagation^[5] and seed germination^[6], and the research on artificial cultivation technology is still in its infancy, and there are few reports on the research on the high-yield fertilization technology of *Pulsatilla chinensis*, and fertilization in *Pulsatilla chinensis* production mostly depends on farmers' experience, resulting in environmental pollution and resource waste. In this study, field experiments were designed with three factors quadratic saturation and D- optimal design, and the effects of nitrogen, phosphorus and potassium formula fertilization on leaf photosynthetic characteristics, dry matter yield and total saponin content of *Pulsatilla chinensis* in growth stage were analyzed in depth, in order to determine the optimal fertilization amount of nitrogen, phosphorus and potassium, and provide theoretical basis for standardized production of *Pulsatilla chinensis*.

2. MATERIALS AND METHODS

2.1. General Situation of the Test Site

The experiment was conducted in the training base of Jiangsu vocational college of agriculture and animal husbandry science and technology (32 27' 42" N, 119 55' 57" E), located in the middle of Jiangsu province, with an average annual temperature of 14.4 °C - 15.1 °C; The average annual precipitation is 1037.7 mm, and the rainfall day is 113 D. the soil type is sandy soil. the 0 - 30 cm plough layer soil contains 23.17 g·kg⁻¹ organic matter, 92.63 mg·kg⁻¹ alkaline hydrolysis nitrogen, 30.37 mg·kg⁻¹ available phosphorus, 92.34 mg·kg⁻¹ available potassium and 6.86 ph.

2.2. Experimental Materials

Pulsatilla seeds were collected from Liuhe district, Nanjing city, Jiangsu province, China. they were

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identified as seeds of Ranunculaceae plants by professor Tang Geng - Guo, Nanjing forestry university. they were sown in the acupuncture points of the training base in Jiangsu vocational college of agriculture and animal husbandry science and technology in early March, 2015. they were planted in the test site in October of the same year. the row spacing of plants was 15 cm× 25 cm, and the plot area was 10 m× 1.5 m. Urea (containing 46 % N) is used as nitrogen fertilizer, superphosphate (containing 14 % P₂O₅) is used as phosphorus fertilizer, and potassium sulfate (containing 54 % K₂O) is used as potassium fertilizer.

2.3. Test Design

This experiment adopts the three-factor quadratic saturation and D - optimal design, selects three kinds of fertilizers, namely nitrogen, phosphorus and potassium, and sets four levels for each fertilizer, CK as the control, and sets 10 treatments in total, and repeats them for three times. see table 1 for specific scheme design. In early April, 2017, all fertilizers were evenly mixed according to the design scheme, furrowing between rows, and top dressing was applied in two times, and other management measures in each community were the same.

Table1. N, P and K three factors quadratic saturation and D - optimal design scheme

Treatment number	Fertilizing amount (kg/hm ²)			Processing number	Fertilizing amount0 (kg/hm ²)		
	N	P ₂ O ₅	K ₂ O		N	P ₂ O ₅	K ₂ O
1	0	0	0	6	107.3	0	134.2
2	180	0	0	7	0	134.2	134.2
3	0	225	0	8	63.8	225	225
4	0	0	225	9	180	79.8	225
5	107.3	134.2	0	10	180	225	79.8

2.4. Test methods

2.4.1. Determination of Photosynthetic Parameters

From April to September, 2017, the fully expanded functional leaves (3rd to 4th leaves from outside to inside) were selected in the middle of each month to measure photosynthetic parameters, and on sunny morning (9: 00 - 10: 00), the fully expanded functional leaves of *Pulsatilla* with different fertilization treatments were measured by ciras - 2 portable photosynthetic measurement system, including net photosynthetic rate (*P_n*), stomatal conductance (*G_s*), intercellular CO₂ concentration (*C_i*) and transpiration rate (*T_r*). During the determination, the light intensity is about 800 μmol.m⁻².s⁻¹, the atmospheric temperature is (25±1°C), and the atmospheric CO₂ concentration is 400±10μmol.mol⁻¹.

2.4.2. Determination of Dry Matter

From April to September, 2017, samples were taken in the middle of each month, and 10 plants were randomly selected for each treatment. after cleaning, the above-ground part was separated from the underground part, and the above-ground part was de-enzyme in an oven at 105 °C for 15 min, and then the underground part was dried to constant weight in an oven at 60 °C together with the underground part, and the dry matter weight was weighed, and the root-shoot ratio was calculated.

2.4.3. Determination of Total Saponin Content Pulverizing Dried Roots of Each Treatment

Sieving with 60 mesh sieve, extracting total saponin by ultrasonic method, and determining total saponin content by vanillin - perchloric acid colorimetry^[7]. Excel 2013 was used for data processing and tabulation, and DPS 9.50 software was used for variance analysis, significance test (Duncan new complex polar difference method) and correlation analysis.

3. RESULTS AND ANALYSIS

3.1. Effects of Different Fertilization Treatments on Photosynthetic Characteristics of *Pulsatilla Chinensis*

As can be seen from table 2, compared with the control (treatment 1), photosynthetic parameters of *Pulsatilla chinensis* leaves in different treatments increased to different degrees, and the differences were significant or extremely significant. Single application of nitrogen fertilizer, phosphate fertilizer and potash fertilizer promoted the net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and transpiration rate of *Pulsatilla chinensis* leaves in detail. among them, single application of nitrogen fertilizer promoted the most obvious effect, followed by single application of

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phosphate fertilizer, and the difference among them reached significant level. The effects of nitrogen, phosphorus and potassium fertilizers on photosynthetic parameters of *Pulsatilla chinensis* leaves were as follows: nitrogen-phosphorus combination > nitrogen-potassium combination > phosphorus-potassium combination. In the treatment of nitrogen fertilizer, phosphate fertilizer and potash fertilizer, the promotion effect of stomatal conductance and transpiration rate in treatment 9 was the most obvious, and the promotion effect of net photosynthetic rate and intercellular CO₂ concentration in treatment 10 was the most obvious.

Table2. Photosynthetic parameters of different fertilization in *Pulsatilla chinensis* ($\bar{x} \pm s$)

Number	(Pn) Photosynthetic rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	(Gs) Stomatal conductance ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	(Ci) Intracellular CO ₂ concentration ($\mu\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	(Tr) Transpiration rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
1	5.45±0.27Cd	67.24±3.23Fe	292.02±11.03De	0.83±0.01Ee
2	6.40±0.25A Bb	109.10±4.12Bb	345.65±12.18A Babc	1.50±0.02A Bab
3	6.01±0.26BCbcd	89.16±2.74DEcd	320.82±10.00A BCDcde	1.26±0.02CDd
4	5.63±0.22BCcd	70.21±4.51Fe	298.71±13.41CDde	0.90±0.02Ee
5	6.33±0.31A Bb	105.37±3.09BCb	339.01±12.91A BCabc	1.50±0.03A Bab
6	6.15±0.13BCbc	96.09±4.17CDc	325.12±10.63A BCDbcd	1.38±0.01BCc
7	5.87±0.21BCbcd	84.16±4.22Ed	319.22±13.29BCDcde	1.21±0.02Dd
8	6.21±0.27A BCb	105.82±5.80BCb	336.73±12.55A BCabc	1.40±0.02BCbc
9	6.42±0.22A Bb	125.00±4.15Aa	352.77±10.53A Bab	1.60±0.02Aa
10	6.99±0.25Aa	114.23±5.06ABb	363.38±15.01Aa	1.53±0.01A Ba

Note: Data presented in the tale were mean values \pm SD of three repetitions in the same treatments. Different capital letters indicated significance at $P < 0.01$ level, small letters indicated significance at $P < 0.05$ level. The same below.

3.2. Effects of Different Fertilization Treatments on the Yield and Total Saponin Content of *Pulsatilla Chinensis*

From table 3, compared with the control (treatment 1), the dry weights of the aerial parts and roots of *Pulsatilla chinensis* in each treatment increased to different degrees, and the differences were significant or extremely significant. Single application of nitrogen fertilizer, phosphorus fertilizer and potassium fertilizer significantly promoted the growth of aboveground parts, among which single application of nitrogen fertilizer promoted the most obvious effect, followed by potassium fertilizer, and finally single application of phosphorus fertilizer. the difference between the three reached significant level. Among the treatments of nitrogen fertilizer, phosphorus fertilizer and potassium fertilizer, the treatment of 9 aerial parts showed the most obvious effect of increasing yield, with 96.30 % increase. In addition, single application of nitrogen fertilizer increased root weight as the most obvious, followed by phosphate fertilizer. Among the treatments of nitrogen fertilizer, phosphate fertilizer and potash fertilizer, the root weight of treatment 10 had the most obvious effect of increasing yield, with 67.86 % increase.

The total saponin content of *Pulsatilla* root in each treatment was significantly higher than that of the control (treatment 1), and the total saponin content increased by 25.07 % - 64.36 %, and there was significant difference among the treatments. The yield increase effect of phosphate fertilizer alone on total saponin content is the most obvious, followed by nitrogen fertilizer and potassium fertilizer alone. The effect of nitrogen fertilizer, phosphate fertilizer and potassium fertilizer on the total saponin content in *Pulsatilla chinensis* was as follows: nitrogen-phosphorus combination > phosphorus-potassium combination > nitrogen-potassium combination. Among the treatments of nitrogen fertilizer, phosphate fertilizer and potash fertilizer, the yield increase is the most obvious among the treatments with 10 total saponin contents.

Table3. Biomass and total saponins contents of different fertilization in *Pulsatilla chinensis* ($\bar{x} \pm s$)

Number	In Aerial parts / DW (g/ each plant)	Roots / DW (g/ each plant)	Root-top ratio	Total saponins (mg/g)
1	10.28±4.15Ff	5.88±2.02Ff	0.57±0.02BCbc	42.17±1.21Ef
2	14.92±3.55Dd	7.86±1.98CEDcd	0.53±0.01CDcd	55.42±0.92CDde

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3	12.05±4.31EFe	7.21±2.31Ede	0.60±0.01A Bb	57.89±0.61 CDcde
4	12.85±3.85Ee	8.39±1.02BCDbc	0.65±0.03Aa	52.96±1.31De
5	17.27±2.90BCb	7.92±1.73CDEcd	0.46±0.02EFfg	60.12±1.73BCDbcd
6	15.55±3.66CDcd	7.48±1.90DEde	0.48±0.01DEFef	52.74±0.88De
7	16.32±4.79CDbcd	6.93±2.54Ee	0.42±0.01Fg	54.77±1.40De
8	16.77±2.68CDbc	8.72±2.88BCb	0.52±0.01CDEde	62.33±1.02ABCbc
9	20.18±4.94Aa	9.06±2.05A Bb	0.45±0.02Ffg	65.20±0.89ABab
10	18.85±3.56ABa	9.87±1.79Aa	0.52±0.01CDEde	69.31±1.07Aa

3.3. Correlation Analysis Between Photosynthetic Characteristics, Quality and Yield of *Pulsatilla Chinensis*

As can be seen from table 4, there is no significant correlation between root removal dry weight and transpiration rate after different nitrogen, phosphorus and potassium fertilization, and there is a significant or extremely significant positive correlation between photosynthetic parameters of *Pulsatilla chinensis* leaves and dry weight of aboveground parts, dry weight of roots and total saponin content, which indicates that the optimization of photosynthetic characteristics of *Pulsatilla chinensis* growth after nitrogen, phosphorus and potassium fertilization improves the yield and quality of *Pulsatilla chinensis*.

4. DISCUSSION AND CONCLUSION

4.1. Effect of Nitrogen, Phosphorus and Potassium Fertilization on Photosynthetic Characteristics of *Pulsatilla Chinensis*

Plant photosynthetic parameters to some extent reflect the growth of plants. Nitrogen, phosphorus and potassium have obvious effects on plant photosynthesis, especially phosphorus. proper phosphorus application can promote the transmission and phosphorylation of photosynthetic electrons, reduce quantum demand and increase net photosynthetic rate [8]. In this study, the photosynthetic parameters of *Pulsatilla chinensis* leaves showed high consistency and had high correlation with the yield and quality of *Pulsatilla chinensis*. it showed that fertilization could improve the effect of " metabolic source" of *Pulsatilla chinensis* leaves, produce more nutrients and finally store them in " metabolic pool", thus improving the yield and quality of *Pulsatilla chinensis*. In addition, in the formula fertilization of nitrogen, phosphorus and potassium, the single application of potassium fertilizer had little influence on photosynthetic parameters of *Pulsatilla chinensis*, which might be related to the higher concentration of available potassium in the soil of this test site (water network area of lixiahe plain).

4.2. Effect of Nitrogen, Phosphorus and Potassium Fertilizer on Yield of *Pulsatilla Chinensis*

In the research of nitrogen, phosphorus and potassium formula fertilization, the research on crop yield is the most concentrated field at present. through formula fertilization, the best fertilization scheme and the best yield can be found. The early balanced fertilization scheme mainly aims to maintain the original fertility level of soil and supplement the nutrient elements consumed by crops, while the modern balanced fertilization scheme develops in depth and diversity, aiming at obtaining larger yield (biomass) on the premise of not affecting the nutrient balance. The results showed that nitrogen fertilizer had the greatest influence on the aboveground part and root yield of *Pulsatilla chinensis*. phosphorus fertilizer had greater influence on the aboveground part yield than potassium fertilizer, while potassium fertilizer had greater influence on root yield than phosphorus fertilizer, which indicated that different parts of *Pulsatilla chinensis* had different requirements on fertilizer. nitrogen and phosphorus combined application might be more beneficial to photosynthesis and growth of aboveground part, while nitrogen and potassium combined application was more beneficial to dry matter accumulation of roots. Huang qiaoyi and other studies found that nitrogen fertilizer had the greatest influence on cassava biomass, followed by potassium fertilizer and phosphorus fertilizer [9]; Li hong and other studies found that nitrogen fertilizer had the greatest influence on *bletilla striata* yield, followed by phosphate fertilizer and potash fertilizer [10], which might be related to different plant species, different research plant organs and different soil and environmental quality in the test site.

4.3. Effects of Nitrogen, Phosphorus and Potassium Fertilizer on the Quality of *Pulsatilla Chinensis*

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In the research of nitrogen, phosphorus and potassium fertilization, the quality of the final harvest products of crops has always been one of the important parameters of the research. The results of this study show that phosphate fertilizer has a great influence on the quality of *Pulsatilla chinensis*. phosphorus is the key factor to realize various functions in plant life, and it helps to store and transport energy. at the same time, it is also one of the main participants of biological substances such as phosphoric acid, phosphoprotein, phospholipid and phosphosugar^[11]. Zhao kai et al studied the effects of nitrogen, phosphorus and potassium on onion yield and quality, and found that phosphate fertilizer had the greatest effect on onion quality, followed by nitrogen fertilizer and potassium fertilizer, which was similar to the results of this study^[12]. To sum up, *Pulsatilla chinensis* is a nitrogen-loving plant, which requires a large amount of nitrogen fertilizer. phosphate fertilizer has a larger influence on photosynthetic characteristics, root growth and quality, and potassium fertilizer has a more obvious influence on the growth of aboveground parts. The precise fertilization research of medicinal plants should pay attention to the yield and the quantity of secondary metabolites at the same time. the balanced application of nitrogen, phosphorus and potassium has a good promoting effect on the yield and quality of *Pulsatilla chinensis*. considering the yield and quality comprehensively, the optimal fertilization scheme is 180 kg / hm² nitrogen, 225 kg / hm² phosphorus and 79.8 kg / hm² potassium, which is worthy of application and popularization.

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