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ORIGINAL PAPER

Effects of nitrogen on growth parameters and photosynthetic characteristics of *P. chinense* Schneid. Seedlings in three soil types*

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Abstract

Soil type and nitrogen (N) are two important factors affecting plant growth and development, and photosynthetic physiological characteristics. However, there are still relatively few studies on the effects of N on *P. chinense* Schneid. seedlings under different types of soils. In this study, three soil types (red soil, alkaline purple soil, and acidic purple soil) and three N levels (50, 150 and 300 mg/plant) were used to investigate how N levels affected seedling growth, biomass accumulation distribution and photosynthetic characteristics under three soil types. The results showed that N may promote the growth of these seedlings and increase their height and basal stem under three soil types. The stem and leaf biomass, aerial biomass, total biomass, and root-shoot ratio showed significant differences. N increased chlorophyll content in leaves on alkaline purple soil and acidic purple soil, but inhibited chlorophyll synthesis on red soil. Under the same N application, the order for total biomass accumulation in seedlings was alkaline purple soil, red soil, and acid purple soil; for the net photosynthetic rate, it was red soil, acid purple soil, and alkaline purple soil. These results indicate that leaves might elevate the photosynthetic rate by increasing stomatal conductance, intercellular CO_2 concentration, and transpiration rate, thereby promoting the growth of seedlings. In addition, soil type and nitrogen had a significant interaction effect on the growth and photosynthetic characteristics of *P. chinense* Schneid. seedlings. These findings will help screening for suitable soil types and nitrogen application levels for efficient cultivation of *P. chinense* Schneid. seedlings.

Keywords: *P. chinense* Schneid., growth, biomass, photosynthetic characteristics

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INTRODUCTION

Nitrogen (N) is one of many nutrients essential for plant growth and development, and for good quality of yield. On the one hand, N promotes growth and development by improving the photosynthetic capacity and nutrient transport capacity of plants. Nitrogen is also the main substance that plays a catalytic role in the physiological metabolic process of plants, obtaining nutrients and promoting growth and development by absorbing and fixing organic and inorganic state N in the soil. In China, N deficiency has become one of the core problems affecting crop yield and quality as most soils have less than 0.2% N due to years of cultivation (Xuan et al. 2017, Luo et al. 2020). N directly and/or indirectly affects the growth, yield and quality of crops. Studies have shown that N significantly increases field survival, relative height growth rate, and relative diameter growth rate of *Larix olgensis* Henry seedlings (Li et al. 2013). N significantly raised above- and below-ground biomass of *Leymu schinensis* seedlings, as well as flowering density, seed number, seed weight, and seed germination (Gao et al. 2020). Moreover, N application significantly increased C and N contents in leaves and roots, C content of stems, N: P ratio of leaves and stems, and C: P ratio of whole roots of *Pinus tabuliformis*. These reports showed that N may promote nutrient uptake in the root system, increase the area of leaf photosynthesis, and enhance the efficiency of dry matter accumulation, which in turn has a significant pro-growth effect on leaves and roots (Jing et al. 2017). However, higher N supply may result in lower photosynthetic N use efficiency, which indicate lower utilization efficiency of accumulated ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) – Li et al. (2018). These studies showed that leaf area index, leaf nitrogen content, intrinsic water use efficiency, and net photosynthetic yield may increase significantly under N applications. N may regulate the carbon allocation pattern in plants, and promote the growth of aerial parts such as leaves and stems, further affect the content and activity of enzymes related to photosynthesis, and also promote chlorophyll synthesis (Xuan et al. 2017, Zhang et al. 2018, Mu, Chen 2021). The differences in N requirements of different plant species and their developmental stages are highly variable, and the intrinsic variation characteristics of how N application affects seedling growth and development and regulates photosynthetic characteristics still need to be further studied.

Soil provides essential mineral elements and water for plant growth and development, and soil varies in physical, chemical and biological properties such as texture, structure, capacity, porosity, soil pH, organic matter, mineral elements, soil fauna and soil microorganisms. These differences directly or indirectly affect plant growth and development and physiological metabolism. Plants growing on different soil types exhibit different biomass accumulation, photosynthetic characteristics, physiological characteristics, and metabolites (Kahkashan et al. 2016). Humus, sandy soil, vegetable garden soil, and yellow-brown loam have different effects on the growth traits and photosynthetic parameters of *Hibiscus sabdariffa*. Biomass, bulb diameter, floral axis height and leaf length, net photosynthetic rate (Pn), chlorophyll content, transpiration rate (Tr), intercellular CO_2 concentration (Ci) and stomatal conductance (Gs) were highest when cultured on humus soil. The best agronomic, physiological, and biochemical characteristics were exhibited by *Lycoris aurea* cultured on humus soil, which may be related to the higher organic matter content, soil enzyme activity, mineral element content such as the alkaline dissolved nitrogen, fast-acting phosphorus and calcium, loose texture and good permeability of humus soil (Quan, Liang 2017). There were significant differences in growth parameters, chlorophyll, total phenols, and total sugars per plant cultured on sandy clay, sandy clay loam, sandy loam, and loamy sand. The highest values were recorded for plants cultured on sandy clay loam (Kahkashan et al. 2016). These reports suggested that plants may be suitable for various soil types, but screening for suitable soil types is one of the important determinants of healthy growth, maximum yield and best quality.

Phellodendron chinense Schneid. (*P. chinense* Schneid.), commonly known as Chuan Huang bai, belongs to the genus *Phellodendron* (Rutaceae) deciduous trees. It is used in Chinese traditional medicine because its stem bark contains alkaloids and other active ingredients. As well as being able to lower blood pressure and blood sugar, these ingredients have anti-viral, anti-inflammatory, anti-cancer, anti-bacterial effects. Moreover, preparations from this plant are used to treat COVID-19 infections (Zhao et al. 2021). Southwest China, including Sichuan, Chongqing, Guizhou, etc., is the main production area of *P. chinense* Schneid. It is widely distributed and planted in a variety of soil types, including yellow soil, purple soil, red soil, alluvial soil, etc. (Sun et al. 2019). The plants may grow and develop normally on slopes, flatlands, and roadsides in mountains and hills from 500 to 2000 m above sea level. Moreover, the types and amounts of secondary metabolites in the stem bark of *P. chinense* Schneid. vary significantly among different production areas, which may be related to ecological differences, especially soil types. The alkaloids content of *P. chinense* Schneid. from Sichuan is higher than in the other production areas in China, and this might be due to the uniqueness of the natural environmental conditions and cultivation techniques. Thus, it has formed a distinctive *P. chinense* Schneid. production area, and enjoys high reputation in China (Tian et al. 2019). For genuine regional drugs, their active pharmaceutical ingredients are related to many factors, such as soil type, light, and humidity in the distribution area. Soil type might be one of the key factors affecting primary and secondary metabolism in medicinal plants (Fang et al. 2020). Although various soil types have been adopted during the extension of the area of cultivation of *P. chinense* Schneid., obtaining high-quality and high-yielding herbs still depends on the most suitable soil type, especially on the cultivation of high-quality seedlings. In this study, the effects of N on growth, biomass accumulation and distribution as well as photosynthetic characteristics of *P. chinense* Schneid. seedlings were investigated on three soil types.

MATERIALS AND METHODS

Materials and reagents

P. chinense Schneid. seeds were collected in December 2018, in the town Xie-yuan, Chengdu, in China, and they were evaluated in the laboratory according to basic indices, based on purity, thousand-grain weight, moisture content, and high viability. Red soil, alkaline purple soil, and acidic purple soil were collected from the village San-xing, and the towns Feng-le, Shi-mian, Yaan, in China (N 29°32′E 102°54′, altitude 878 m), the town Ji-feng, Deyang, in China (N31°03′E 104°68′, altitude 900 m), and from Lao-banshan Reading Park, Ya-an, in China, (29°58′N, 102°58′E, altitude 679 m), respectively. These soils were naturally air-dried, ground, picked to remove impurities, and mixed, and basic indices were determined. The basic physico-chemical properties such as pH and concentrations of total carbon (C), nitrogen (N), phosphorus (P), and potassium (K) were analyzed prior to the experiment (Table 1). Soil pH was analyzed in water extracts (deionized water: soil ratio 5:1) with a PB-10 pH meter (Sartorius GmbH, Göttingen, Germany) after shaking for 1 h. The C content was determined by potassium dichromatate oxidation and external heating method. The measured liquid solutions with N, P, K were prepared with concentrated H_2SO_4-$HClO_4$. The N and P levels were determined using the semimicro-Kjeldahl method, and the Mo-Sb content was assayed with the colorimetric method. The K content was determined by atomic absorption spectrophotometry (Chen et al. 2015).

Overview of the study area

The test site is the fifth teaching building of Sichuan Agricultural University, District Wenjiang, Chengdu, China (30°38′N, 103°45′E). The city

Table 1

Basic chemical properties of three types of soil

Specification	Red soil	Alkaline purple soil	Acid purple soil
pH	5.75±0.075	8.07±0.105	6.57±0.044
Organic carbon (g kg^{-1})	33.89±0.64	33.24±0.74	28.51±0.66
Total nitrogen (g kg^{-1})	0.42±0.07	0.55±0.15	0.89±0.09
Total phosphorus (g kg^{-1})	0.37±0.03	0.68±0.01	0.63±0.07
Total Potassium (g kg^{-1})	8.84±0.08	19.18±0.07	12.17±0.10
Bulk density (g cm^{-3})	1.30±0.06	1.25±0.08	1.29±0.04

lies in the mid-latitude inland subtropical monsoon climate with mild weather conditions, with four distinct seasons, and at the altitude of 510~600 m. The annual average rainfall is 896.1 mm, with the wettest season of the year from June to September; the annual average temp. is 16.4°C; the annual average relative humidity reaches 84%; the annual average sunshine hours is 1104.5 h, and the annual frost-free period is 282 days.

Seed germination and seedling culture

Seeds were soaked in 0.1% $KMnO_4$ for 30 min for disinfection and sterilization, and then transferred to distilled water for 3 days until they turned white. The water was changed every day, and then these seeds were germinated in a box with nutrient soil, maintained at 25°C. The seeds began to sprout after 15 days, and were transplanted when the seedlings grew to about 5 cm tall. Before transplanting, 0.2 g of calcium superphosphate and 0.1 g of potassium sulfate were added to each pot as the base fertilizer. The seedlings were planted into pots containing 5 ± 0.5 kg of different soil types (pot diameter 37 cm, pot height 27 cm). The study followed a split-plot randomized design of an experiment. It contained four nitrogen levels: CK – 0 mg/plant, T1 – 0 mg/plant, T2 – 150 mg/plant, T3 – 300 mg/plant, and three soil types: red soil, alkaline purple soil and acidic purple soil. The experiment consisted of 12 treatments and 9 replicates per treatment and a total of 108 pots. After 20 days, the seedlings were treated with NH_4NO_3 needed for the three nitrogen levels (0, 50, 150 and 300 mg/plant). The seedlings were cultivated in a greenhouse, where the presence of insects and weeds was prevented, and watered regularly every day to maintain normal growth during the growth period.

Determination of growth index and biomass

Seedling height (cm) and basal stem (mm) in each groups were measured on June 1, June 17, July 3, July 19, and August 4, 2019. Seedling height growth (ΔSH, cm) – seedling height measured on each occasion (cm) – seedling height measured on June 1 (cm), basal stem growth (ΔSBD, mm) – basal stem measured on each occasion (mm) – basal stem measured on June 1 (mm). In August 2019, after the net photosynthetic rate measurement, three plants per soil type were selected, dug out with roots, rinsed in deionized water and dried off the surface water, labeled in kraft paper bags by root, stem, and leaf, killed in the oven at 105°C for 30 min, adjusted to 65°C and baked to constant weight, weighed on an electronic balance, and submitted to calculations.

Determination of photosynthetic indices

The leaf gas exchange rate was measured by using a photosynthesis meter Li-CorInc., USA. To ensure that the measurement environment approximated the ideal photosynthetic state, measurements were carried out from 9:00 to 11:00 a.m. on a sunny day with a manually controlled CO_2 concentration of 400 μmol mol^{-1}, leaf surface temp. of 25°C, and light intensity of 1500 μmol m^{-2} s^{-1}. The first complete round of functional leaves under the top shoot of seedlings was selected to determine the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular carbon dioxide concentration (Ci), and to calculate instantaneous water use efficiency (WUE, WUE=Pn/Tr), with three plants per group and three replicates per plant. The contents of pigment were measured according to the method of Lichtenthaler and Wellburn (1983). The leaves used for gas exchange rate determination were cut, wrapped in tin foil, brought back to the laboratory, washed with deionized water and dried, then cut up, and weighed 0.1 g in 10 ml of extraction solution (80% acetone: anhydrous ethanol: water = 4:5:1), sealed and stored away from light. After these leaves were extracted until they were completely white, the supernatant was taken and its absorbance values at 663 nm, 646 nm, and 470 nm were measured using a UV spectrophotometer.

Statistical analysis

Microsoft excel 2010 was used to process the experimental data. The results of multiple comparisons between means were tested using the Duncan test (P<0.05) and One-way ANOVA was performed by SPSS Statistics 19.0. The analysis of soil type, nitrogen levels, and their interaction was performed by Univariate multi-factor ANOVA (P<0.05). Correlation analyses were also performed for each physiological index, and the results were plotted in OriginPro 9.0.

RESULTS

Effects of N on seedling height and basal stem under three soil types

Figure 1 shows that N increased seedling height (ΔSH) and basal stem growth (ΔSBD) in *P. chinense* Schneid. seedlings. The degree of effects of different N levels on ΔSH and ΔSBD varied on the three soil types. On red soil, ΔSH and ΔSBD reached the maximum at T3 level, being significantly higher, by 60.16% and 34.71% respectively, than in the CK group (P<0.05). On alkaline purple soil, ΔSH was significantly higher (P<0.05) than the same parameter in the CK group at all N levels from July onwards, and ΔSH at T2 level was the highest during the growth stage. Under different N levels, ΔSBD was significantly higher (P<0.05) than that in the CK group. On acidic purple soil, only ΔSH under T2 level was significantly higher than that in the CK group, by 38.40% (P<0.05), while ΔSH under the other N levels was not significantly different from the values obtained in CK group throughout the growth period (P>0.05). ΔSBD under T1 level was significan-

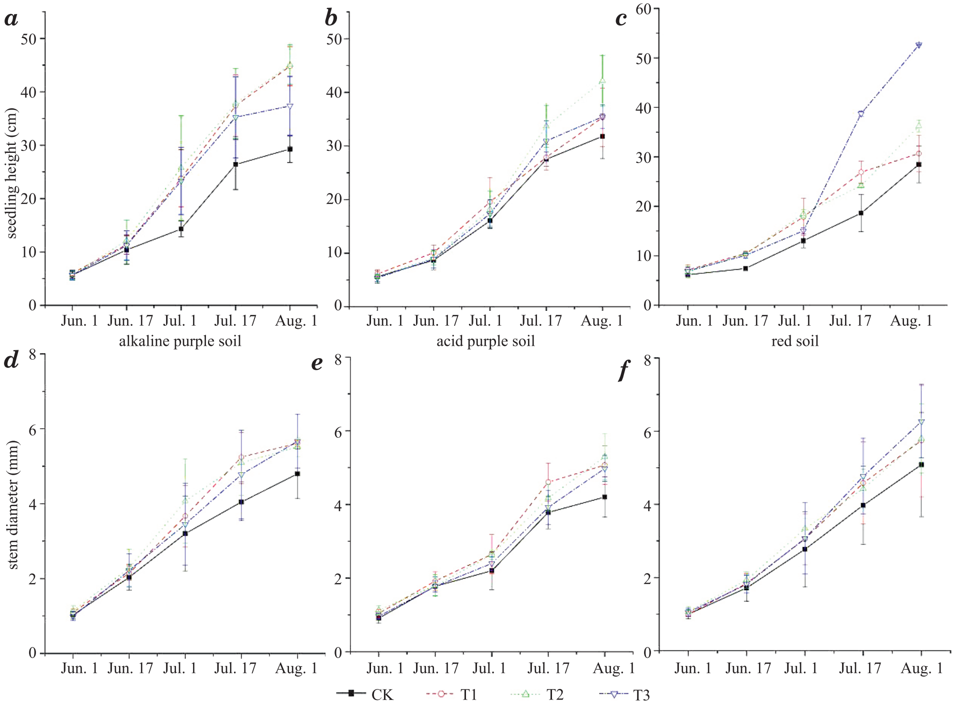

Fig. 1. Effects of N on seedling height and stem basic diameter of *P. chinense* Schneid. seedlings under three soil types. CK – 0 mg per plant, T1 – 50 mg per plant, T2 – 150 mg per plant, T3 – 300 mg per plant

tly higher than that of in the CK group by 28.92% (P<0.05), respectively, and ΔSBD under the other N levels were not significantly different from the one in the CK group throughout the growth period (P>0.05).

Effects of N on biomass accumulation and distribution under three soil types

As shown in Table 2, N promoted an increase in seedling biomass on the three soil types. On each soil type, different N significantly increased total biomass (TB), aerial biomass (AGB), biomass (SB), and leaf biomass (LB) of seedlings compared to CK (P<0.05). On red soil, TB level was the largest at T3, increasing 1.46-fold compared to the CK group, but the effect of N on the root-shoot ratio (R/S) did not reach a significant level (P>0.05). On alkaline purple soil, the biomass of all organs reached the maximum at T2 level. In addition, RB was significantly lower than in the CK group, by 14.00% at T3 level. On acidic purple soil, seedlings reached the maximum biomass, and the root-shoot ratio of all organs at T2 level as well as TB increased 1.38 times compared to CK (P<0.05).The effects of N on biomass accumulation and distribution on the three soil types are shown in Figure 2. Overall, N reduced root weight ratio (RWR) on red soil and alkaline purple

Table 2

Effects of N on biomass accumulation under three soil types

Soil type	N levels	Leaf biomass (g)	Stem biomass (g)	Root biomass (g)	Aboveground biomass (g)	Total biomass (g)	Root/Shoot ratio
Red soil	CK	2.61±0.34*d*	0.97±0.02*f*	2.18±0.06*d*	3.58±0.32*e*	5.76±0.34*e*	0.62±0.05*a*
	T1	3.77±0.15*c*	1.83±0.05*e*	2.54±0.07*c*	5.59±0.20*d*	8.13±0.24*d*	0.45±0.02*b*
	T2	5.17±0.10*a*	3.03±0.06*c*	3.24±0.14*b*	8.21±0.12*b*	11.44±0.25*b*	0.39±0.01*b*
	T3	5.21±0.06*a*	4.82±0.11*a*	4.15±0.11*a*	10.03±0.17*a*	14.18±0.26*a*	0.41±0.01*b*
Alkaline purple soil	CK	3.24±0.07*d*	1.53±0.01*f*	2.91±0.05*c*	4.77±0.07*e*	7.68±0.09*f*	0.61±0.01*a*
	T1	4.05±0.14*c*	2.63±0.03*e*	2.78±0.01*c*	6.67±0.16*d*	9.45±0.18*e*	0.42±0.01*b*
	T2	7.14±0.10*a*	5.22±0.06*a*	4.11±0.05*a*	12.36±0.06*a*	16.47±0.07*a*	0.33±0.00*c*
	T3	4.06±0.23*c*	3.64±0.07*c*	2.50±0.05*d*	7.70±0.30*c*	10.20±0.35*d*	0.32±0.01*c*
Acid purple soil	CK	2.39±0.18*e*	1.43±0.04*e*	1.67±0.22*d*	3.81±0.15*e*	5.48±0.25*f*	0.44±0.06*ab*
	T1	3.66±0.11*d*	1.79±0.06*d*	2.57±0.08*b*	5.44±0.06*d*	8.01±0.07*e*	0.47±0.02*a*
	T2	5.77±0.08*a*	3.43±0.21*a*	3.87±0.10*a*	9.20±0.15*a*	13.07±0.11*a*	0.42±0.02*ab*
	T3	4.53±0.09*bc*	2.14±0.05*c*	2.08±0.03*c*	6.67±0.11*c*	8.75±0.15*d*	0.31±0.00*c*

Different letters indicate significant difference in different N levels on same soil type (P<0.05). CK, T1, T2, T3 represented 0, 50, 150, and 300 mg per plant, respectively.

soils, and stem weight ratio (SWR) tended to increase significantly (P<0.05) with increasing N levels.

Effect of N on pigment content under three soil types

As shown in Table 3, the total Chl content on red soil was significantly reduced by 17.00% and 16.70% at T1 and T2 levels compared with the CK (P<0.05), respectively. However, Chl a/b was significantly increased (P<0.05), and N inhibited carotenoid synthesis in leaves. On alkaline purple and acidic purple soils, N promoted an increase in the pigment content in leaves to different degrees, reaching the maximum at T2 level. On alkaline purple soil, N significantly promoted leaf Chl a synthesis (P<0.05), and the maximum content of Chl a, Chl b, Caro, and total Chl at T2 level, with significant increases of 22.16%, 37.74%, 15.00%, and 26.36% compared to the CK group (P<0.05), respectively. On acidic purple soils, the pigment content was higher than in plants on both red and alkaline purple soils at the same level of N, and the Chl a, Chl b, and total Chl content was significantly (P<0.05) increased at T1 level compared to the CK group.

Effects of N on gas exchange parameters under three soil types

As shown in Table 4, N increased leaf net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular carbon dioxide concentration (Ci), and transpiration rate (Tr) to different degrees under three soil types. On red soil, T1 and T2 levels significantly increased leaf Pn, Gs, Ci, and Tr

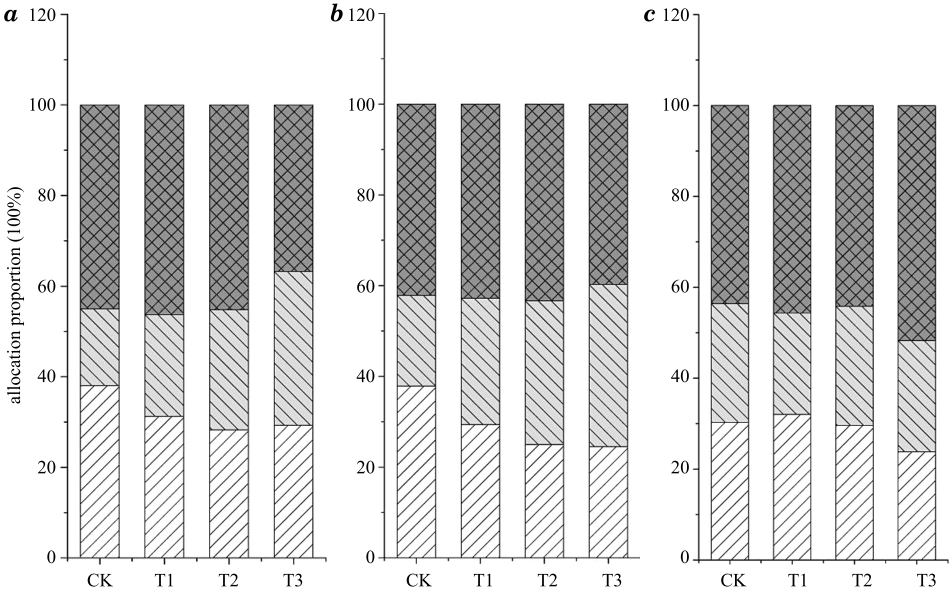

Fig. 2. Effects of N on biomass allocation proportion of *P. chinense* Schneid. seedlings under three soil types: *a*, *b* and *c* – red soil, alkaline purple soil and acid purple soil, respectively, CK, T1, T2, T3 – 0, 50, 150, and 300 mg per plant, respectively

Table 3

Effects of N on pigments content in leaves under three soil types

Soil type	N levels	Chl a (mg g-1)	Chlb (mg g-1)	Caro (mg g-1)	Chl a/b	Total Chl
Red soil	CK	2.11±0.03*a*	0.81±0.02*a*	0.50±0.01*a*	2.58±0.06*b*	2.96±0.06*a*
	T1	1.85±0.04*b*	0.61±0.03*b*	0.45±0.01*cd*	3.06±0.08*a*	2.46±0.07*b*
	T2	1.85±0.02*b*	0.61±0.01*b*	0.45±0.01*de*	3.01±0.04*a*	2.47±0.03*b*
	T3	2.09±0.01*a*	0.78±0.01*a*	0.47±0.00*bc*	2.67±0.03*b*	2.88±0.02*a*
Alkaline purple soil	CK	1.67±0.01*b*	0.53±0.00*c*	0.40±0.00*c*	3.14±0.01*a*	2.20±0.01*b*
	T1	1.89±0.01*a*	0.63±0.01*b*	0.46±0.00*a*	3.02±0.02*ab*	2.51±0.01*a*
	T2	1.87±0.02*a*	0.65±0.01*ab*	0.46±0.00*a*	2.90±0.04*bc*	2.52±0.02*a*
	T3	1.91±0.13*a*	0.72±0.06*ab*	0.44±0.02*ab*	2.69±0.07*d*	2.63±0.19*a*
Acid purple soil	CK	2.09±0.04*c*	0.80±0.04*b*	0.45±0.01*c*	2.62±0.07*a*	2.88±0.08*b*
	T1	2.18±0.03*ab*	0.90±0.03*a*	0.46±0.01*bc*	2.44±0.07*ab*	3.08±0.06*a*
	T2	2.14±0.02*abc*	0.84±0.04*ab*	0.47±0.01*abc*	2.48±0.07*ab*	3.02±0.04*ab*
	T3	2.10±0.02*bc*	0.82±0.02*ab*	0.49±0.00*ab*	2.56±0.06*ab*	2.92±0.03*b*

Different letters indicate significant difference in different N levels on same soil type ($P<0.05$). CK, T1, T2, T3 represented 0, 50, 150, and 300 mg per plant, respectively.

($P<0.05$), and the maximum was observed at T1 level. N significantly suppressed the instantaneous water use efficiency (WUE) – $P<0.05$, and the minimum was observed at T1 level. On alkaline purple soil, the values of Pn,

Table 4

Effects of N on photosynthetic rate in *P. chinense* Schneid. leaves under three soil types

Soil type	N levels	Pn (μmol m^{-2} s^{-1})	Gs (μmol·m^{-2} s^{-1})	Ci (mol m^{-2} s^{-1})	Tr (μmol mol^{-1})	WUE (μmol mmol^{-1})
Red soil	CK	8.09±0.39^{d}	0.07±0.01^{f}	210.8±2.95^{d}	2.81±0.06^{d}	2.87±0.08^{a}
	T1	16.37±1.04^{b}	0.30±0.02^{b}	295.8±5.72^{b}	7.41±0.62^{b}	2.22±0.05^{bc}
	T2	12.43±0.20^{c}	0.15±0.01^{d}	255.0±6.76^{c}	5.52±0.30^{c}	2.27±0.15^{bc}
	T3	8.59±0.19^{d}	0.10±0.00^{e}	364.0±4.76^{a}	3.49±0.02^{d}	2.46±0.04^{b}
Alkaline purple soil	CK	4.60±0.07^{c}	0.04±0.01^{b}	197.2±7.77^{c}	1.85±0.06^{c}	2.49±0.05^{c}
	T1	5.82±0.23^{b}	0.05±0.00^{b}	203.2±10.79^{bc}	1.82±0.08^{c}	3.20±0.16^{a}
	T2	9.75±0.35^{a}	0.10±0.01^{a}	230.2±6.25^{ab}	3.24±0.08^{b}	3.01±0.06^{ab}
	T3	4.13±0.30^{c}	0.04±0.01^{b}	231.3±8.51^{ab}	1.45±0.09^{d}	2.85±0.06^{b}
Acid purple soil	CK	6.49±0.63^{b}	0.04±0.01^{e}	187.8±3.74^{c}	1.93±0.13^{e}	3.34±0.09^{ab}
	T1	9.95±0.52^{a}	0.07±0.00^{cd}	188.1±3.22^{c}	2.88±0.04^{d}	3.45±0.14^{a}
	T2	11.33±0.10^{a}	0.10±0.00^{b}	207.4±2.71^{bc}	3.73±0.06^{b}	3.04±0.07^{bc}
	T3	8.15±0.09^{b}	0.06±0.00^{d}	207.0±4.63^{bc}	2.82±0.08^{d}	2.89±0.05^{c}

Different letters indicate significant difference in different N levels on same soil type ($P<0.05$). CK, T1, T2, T3 represented 0, 50, 150, and 300 mg per plant, respectively.

Gs, Ci, and Tr were significantly higher than in the CK group at T2 level ($P<0.05$), and instantaneous WUE significantly increased by 0.71, 0.52, and 0.36 μmol mmol^{-1} compared to the CK group at three levels of N ($P<0.05$). On acidic purple soil, the Pn was significantly higher than in the CK group at T1 and T2 levels ($P<0.05$), respectively. Compared with the CK group, the instantaneous WUE of leaves increased by 0.11 μmol mmol^{-1} at T1 level ($P>0.05$), and decreased by 0.45 μmol mmol-1 at T3 level ($P<0.05$). In addition, leaf Pn, Gs, Ci, and Tron red soil were higher than on alkaline purple soil and acidic purple soil at the same N level, while the instantaneous WUE on acidic purple soil was higher than on red soil and alkaline purple soil.

Correlation analysis

As shown in Table 5, N, soil type and their interaction had highly significant effects on the biomass accumulation and distribution in different organs ($P<0.001$). However, N had no significant effect on the leaf weight ratio (LWR) – $P>0.05$. As shown in Table 6, soil type had highly significant effects on chlorophyll content, chlorophyll b content, carotenoid content, chlorophyll a/b, total chlorophyll, Pn, Gs, Ci, and Tr, and instantaneous WUE ($P<0.001$). The N level had highly significant effect on Pn, Gs, Ci, and Tr, and instantaneous WUE ($P<0.001$), and its significant effect was also observed on the chlorophyll content in leaves ($P<0.05$). The interaction between soil type and N had highly significant effects on Pn, Gs, Ci, Tr and instantaneous WUE ($P<0.001$), and a significant effect on the chlorophyll a

Table 5

The significance test for the effects of soil types, N and the interaction on the growth indexes of *P. chinense* Schneid. seedlings

Growth indexes	TB	RB	AGB	SB	LB	RWR	SWR	LWR	R/S
P:F_S	***	***	***	***	***	***	***	***	***
P:F_N	***	***	***	***	***	***	***	ns	***
P:$F_{S×N}$	***	***	***	***	***	***	***	***	***

ns, *, **, ***, represent for P>0.05, 0.01<P≤0.05, 0.001<P≤0.01, P≤0.001, respectively.
F_S, soil types effect, F_N, N effect; $F_{S×N}$, the interaction effects of soil types and N.

Table 6

The significance test for the effects of soil types, N and the interaction on photosynthetic characteristics of *P. chinense* Schneid. seedlings

Photo-synthetic indexes	Chl a	Chl b	Carotenoids	Chl a/b	Total Chl	Pn	Gs	Ci	Tr	WUE
P:F_S	***	***	***	***	***	***	***	***	***	***
P:F_N	*	ns	ns	ns	ns	***	***	***	***	***
P:$F_{S×N}$	*	*	*	ns	*	***	***	***	***	***

ns, *, **, ***, represent for P>0.05, 0.01<P≤0.05, 0.001<P≤0.01, P≤0.001, respectively.
F_S, soil types effect, F_N, N effect, $F_{S×N}$, the interaction effect of soil types and N.

content, chlorophyll b content, carotenoid content, total chlorophyll, and net photosynthetic rate in leaves (P<0.05).

DISCUSSION

As the market demand for *P. chinense* Schneid. is dramatically increasing, it is difficult to increase the yield significantly due to the harvesting period of 8~10 years, and therefore artificial cultivation has become the only way to satisfy the market demand and achieve sustainable use of *P. chinense* Schneid. Although *P. chinense* Schneid. may grow normally on a variety of soil types and adapt to various soil environment during the cultivation, it will be an effective high-yield and high-quality method to select suitable soil type for its cultivation. For medicinal plants, the soil type is one of the main factors affecting the medicinal components. Different types of soils have different physicochemical and biological properties, which have a significant impact on the growth, development, and active ingredients of medicinal plants (Kahkashan et al. 2016). Thus, the screening of suitable cultivation areas especially how to cultivate high-quality seedlings are the key issues that need to be addressed in order to expand the planting area of *P. chinense* Schneid..

Different types of soil directly or indirectly affect the exchange of water, air and nutrients between plants and soil, thus influencing the plant growth, development and physiological metabolism. Reports showed that carrots grown on peat soils had better flavor, possessing lower bitterness, earthiness but higher sweetness as well as juiciness, with the lowest tendency to split determined in carrots grown in sandy soils (Seljåsen et al. 2012). Grapes cultivated on wind-sand and chert soils ripened faster than grapes grown on irrigated silt soils, and those grapes grown on wind-sand soils had the highest sugar content, total soluble solids, sugar-acid ratio, and anthocyanin content (Wang et al. 2015). Paula et al. showed that different soil types affect *Ebenopsis ebano* seedling survival and growth, with the best performing soil type being 50% topsoil, 50% vermiculite mixture (Paula et al., 2021). In the present study, the root, stem, leaf and total biomass of seedlings on alkaline purple soils were higher than on acid purple and red soils at the same N levels, which may be related to the physicochemical properties of alkaline purple soils. The alkaline purple soil pH of 8.07 may be one of the factor for higher biomass. Moreover, differences in organic carbon, nitrogen and phosphorus in the three soil types may also be influential factors affecting seedling growth traits, biomass accumulation and distribution (Table 1). Plant growth and development depend on the synthesis and accumulation of organic matter produced by photosynthesis (Quan, Liang 2017). Pn, Gs, Ci and Tr are important indicators characterizing photosynthesis, and their magnitude may directly reflect the photosynthetic capacity of plant, which in turn determines plant growth and productivity (Yan et al. 2019). Under simulated acid rain conditions, Pn and Tr of *Jatropha curcas* on yellow-brown and purple soils were relatively higher than those of on red and yellow soils.WUE was relatively lower than those of on red and yellow soils, and stomatal conductance was the highest in yellow-brown soils (Shu et al. 2019). In *Ligustrum robustum*, the average daily Pn and Tr were the largest on limestone, and the smallest on coal-based sand shale, while the average daily Pn values were significantly higher than on other soils (Yan et al. 2019). The present study showed that the values of Pn, Gs, Ci, and Tr on red soil were higher than on alkaline purple soil and acid purple soil at the same N level, while the instantaneous WUE of leaves on acid purple soil was higher than on red soil and alkaline purple soil. These findings will help to select suitable soil types for the seedling cultivation of *P. chinense* Schneid., and provide data for designing a reasonable fertilization method during the production of seedlings. The current study only tested the influences of three soils types on the growth and photosynthetic characteristics of *P. chinense* Schneid. seedlings. However, the effects on seedling growth and development are long-term and complex, and further long-term localized observations are needed.

Some studies have been conducted on plant seedling N application techniques and differences in N application between different soil types, and diverse results and opinions have been formed. The differences in N application are closely related to soil type, plant development period, plant species, etc. (Moore et al. 2014, Liu et al. 2019). N is an important limiting factor in that the application of high doses of nitrogen (15 g/plant) promotes growth and maximizes biomass production during the early morphological establishment stage of *Pongamia pinnata* (Sahoo et al. 2021). In the present study, results showed that N application increased the height and basal stem of *P. chinense* Schneid. Seedlings, and significantly increased whole plant biomass and leaf biomass on three types of soils. The biomass reached its maximum at T2 level on red soil, and at T3 level on acidic purple and alkaline purple soils, respectively (Figure 1 and 2). This may be due to that red soil had 0.42% N concentration, which was the lowest N content compared to the other soils (Table 1). Different types of soil are vary in texture, structure, absorption performance, pH, nutrient status, etc. Among essential macronutrients, the content of N is one of the key factors affecting growth and development in plant. The correlation between growth and nitrogen use efficiency in some plants has been reported (Pinto et al. 2008, Wei et al. 2010). In addition, seedlings may grow normally on three soil types, but there were differences in growth indices and photosynthetic characteristics (Tables 3 and 4), which indicated that *P. chinense* Schneid. seedlings may adapt to environmental changes by changing biomass allocation and photosynthetic physiological characteristics, and thus adjusting nutrient acquisition strategies. An earlier report showed that N application might improve the uptake and assimilation of nitrogen by *Alhagi sparsifolia* Shap., allowing biomass and nutrients to be allocated to leaves and fine roots, and the root-shoot ratio increased with the amount of N applied (Zhang et al. 2020). The current study showed that the biomass of above- and below-ground parts of *P. chinense* Schneid. seedlings increased with increasing N addition on red soil and alkaline purple soil, and the root shoot ratio decreased. This may be related to the promotion of all organs with N addition (Table 2). Research has been done on the influence of nitrogen on the chlorophyll content and photosynthesis in some plant species (Xuan et al. 2017, Luo et al. 2020). Liang et al. showed that N application significantly increased the photosynthetic rate, stomatal conductance and transpiration rate per unit area in leaves, and the response of the photosynthetic rate was positively correlated with the response of the stomatal conductance (Liang et al. 2020). The present results suggest that N addition may increase the chlorophyll content, Pn, Gs and Ci in leaves on alkaline purple soil and acidic purple soil, and the increase of the chlorophyll content and gas exchange parameters showed a decreasing trend with the increasing N level (Table 2). This may be due to the high N content of leaves that causes the lack of other mineral elements related to chlorophyll syntheses, such as Mg and Mn, further resulting in nutrient imbalance. As a result, chlorophyll synthesis is hindered and thus the increase in photosynthetic capacity decreases. Photosynthesis is a very complex process, which is related to ecological factors, plant species and development stages (Brown et al. 1996; Peuelas

2013). The correlation analysis showed that the soil type and N had highly significant effects on Pn, Gs, Ci, and Tr and instantaneous WUE (P<0.001), while their interaction had highly significant effects on Gs, Ci, and Tr, instantaneous WUE (Table 6). The differences in pH, soil nutrients, and water retention capacity of alkaline purple soil, acidic purple soil, and red soil, as well as the differences in N requirements of different soil types, which directly or indirectly result in corresponding changes in seedlings' physiological activities due to changes of the growth environment. Therefore, *P. chinense* Schneid. seedlings develop adaptations to differential N application levels by regulating their growth traits, biomass accumulation and distribution, and photosynthetic physiological characteristics under three soil types.

CONCLUSIONS

The present results showed that N effectively increased seedling height and ground diameter, and affected biomass accumulation and distribution, photosynthetic characteristics in *P. chinense* Schneid. seedlings on three soil types. The changes in biomass allocation patterns and photosynthetic characteristics also reflect the adaptive capacity and adaptation pathways of *P. chinense* Schneid. seedlings. In addition, N differs significantly among three types of soils due to differences in physicochemical properties, nutrients, and water retention capacity. Therefore, much work is still needed to solve the questions how to adjust the N application level and select suitable soil types during the culture of *P. chinense* Schneid. seedlings. Further studies will focus on the fertilizer requirement characteristics of seedling development stages and the physical, chemical and biological properties of soils for fertilizer management, and to explore the relationship between fertilizer application and seedling quality and metabolites on different types of soils.

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