

PHOTOSYNTHETIC PHYSIOLOGICAL CHARACTERISTICS OF FOUR MEDICINAL PLANTS

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Abstract. The objective of this research was the study of research on the photosynthetic characteristics of four kinds of medicinal plants, to provide a reliable reference for the high quality cultivation of these four plants. Methods: the four kinds of medicinal plants were as the experimental materials. The photosynthetic rate, chlorophyll content, carotenoid content and photosynthesis of four medicinal plants were determined. The effects of net photosynthetic rate on photosynthetic effective radiation were studied, and the water use efficiency and transpiration rate were calculated. Results: through the comparison of net photosynthetic rate, it can be seen that the average photosynthetic rate, chlorophyll content and carotene content of the four medicinal plants are very different in the growing season. Conclusion: *Platycodon grandiflorum* has a wide range of adaptability to light intensity. It can not only utilize intense light efficiently, but also has shade tolerance. It is suggested to be cultivated in an intense light environment; *North Atractylodes* has high light utilization rate, but poor shade tolerance, it is suitable for cultivation in direct sunlight environment; *Carthamus tinctorius* and *Mentha haplocalyx* has strong shade tolerance, but are not resistant to intense light, and can adopt intercropping.

Keywords: medicinal plants, photosynthetic rate, content of photosynthetic pigment, physiological and ecological factors, water use efficiency

Introduction

Traditional Chinese medicine is an important weapon in the prevention and treatment of diseases in Chinese traditional medicine. It has been playing an important role in protecting health. Medicinal plants contain bioactive ingredients, is used for special economic crops and anticorrosion treatment (Kannan et al., 2018). As an important industrial raw material, it can be processed into drugs, pesticides and veterinary drugs (Qadir et al., 2018; Khosravi et al., 2018). China is the country with the largest species of medicinal plants in the world, and has a long history of cultivation. Because medicinal plants are natural, stable, with little toxic and side effects, they have the unique advantages that Western medicine does not have. Modern people are increasingly turning their attention from synthetic chemicals to natural medicines, and hoping to reduce the incidence of drug-induced diseases (Liu et al., 2014).

With the vigorous development of the pharmaceutical industry, the establishment of plant research institutes has been established (Kim et al., 2018; Allahdadi et al., 2018). The production of Chinese herbal medicines has reached an unprecedented level from the variety and scale (Zhu et al., 2014). Unlike the field crop production, the economic characters such as yield and quality of Chinese herbal medicines are not easy to control, and their adaptability is poor. Moreover, because of the “absolute heterogeneity” of

natural plants, the quality evaluation of Chinese herbal medicines is more difficult. Therefore, it is of great theoretical and practical significance (Huang et al., 2016) to study the fertility laws and various physiological characteristics of traditional Chinese medicine. At present, standardized construction of traditional Chinese medicine production bases, breeding and utilization of germplasm resources and excellent varieties, and pesticide residues and heavy metals are all actively studied. However, there are few reports about the relationship between photosynthetic performance and diurnal variation of photosynthesis and ecological factors, especially the research on main limiting factors and physiological characteristics of photosynthesis in different medicinal plants.

Photosynthesis is the process of transforming inorganic matter into organic matter and converting light energy into chemical energy. It is the basis of plant matter production (Lee et al., 2016). Photosynthetic rate determines the ability of photosynthesis, and is the key to the formation of plant biomass. The photosynthetic rate increases with the increase of light intensity in a certain range.

In this study, for further understanding of medicinal plant photosynthesis and physiological changes, the photosynthetic and physiological characteristics and environmental factors of four kinds of medicinal plants (*Platycodon grandiflorum*, *North Atractylodes*, *Carthamus tinctorius* and *Mentha haplocalyx*) are determined and analyzed, and the diurnal changes of net photosynthetic rate and the content of photosynthetic pigments (Chlorophyll a, b, and carotenoid) were measured. Through light saturation point, light compensation point and photosynthetic pigment content, the shade tolerance and photosynthetic capacity were determined, the response of net photosynthetic rate to photosynthetically active radiation was discussed, the transpiration rate and water use efficiency were calculated as well as the physiological effects of ecological factors on the diurnal variation of net photosynthetic rate. The photosynthetic and physiological characteristics of these four medicinal plants are further studied to provide experimental basis for the creation of the cultivation environment which is beneficial to improve its photosynthesis.

Materials and methods

Materials

Brief introduction of materials

Platycodon grandiflorum: a perennial herb of the family Platycodon, and it grows in the shrubs region of less than 2100 m above sea level. It has the effects of relieving sore throat, and eliminating phlegm, etc. (Kim et al., 2016; Wanget al., 2015). *Platycodon grandiflorum* is not only a kind of resource plant but also a kind of economic plant for medicine and food. As shown in *Figure 1* of *Platycodon grandiflorum*.

North Atractylodes: is a perennial herb of family Compositae, mainly divided into the North Atractylodes and the South Atractylodes. The North Atractylodes is mainly distributed in the north of China. It grows on the dry slopes of 300-900 m above sea level, with sparse broad-leaved forests or coniferous broad-leaved mixed forests (Gao et al., 2017; Shen et al., 2017). The site is the main use of *Atractylodes* rhizome, with eliminating heat and removing sweat, digestion and other effects, so the treatment used to ease bloating, diarrhea and edema disease, night blindness, rheumatism and diabetes and so on. As shown in *Figure 2* of *North Atractylodes*.



Figure 1. *Platycodon grandiflorum*



Figure 2. *North Atractylodes*

Carthamustinctorious: is an annual herbaceous plant of family Compositae. It has special fragrance, warm and spicy taste, it has the effects of activating blood circulation, removing blood stasis, dysmenorrhea and relieving pain. It also has the effects of anti-inflammatory, improving myocardial blood supply, anti-aging and protecting liver. Safflower yellow is also a natural quality food pigment and dyestuff. As shown in *Figure 3* of *Carthamustinctorious*.



Figure 3. *Carthamustinctorious*

Mentahaplocalyx: is a perennial herb of the family Lamiaceae. Mint can grow in a region of less than 2100 m above sea level. It has the function of evacuating the wind and heat, clearing the head, and regulating the flow of vital energy and remove obstruction to it and relieving depression. It is good for preventing and curing cholesterol calculus, and has a role in central nervous system and digestive system. It is

mainly for treating wind heat and common cold, headache and stuffiness, throat swelling and pain, rubella and itching. As shown in *Figure 4* of *Mentahaplocalyx*.



Figure 4. *Mentahaplocalyx*

Experimental materials

Study area survey: the test was carried out in the agricultural science and technology demonstration base of Tongzhou District Agricultural Technology Extension Station in Beijing. This area is a continental monsoon climate zone, the annual average temperature is 11.3 °C, the annual rainfall is 620 mm, and the annual sunshine is 2730 h. The soil fertility is medium, and the texture is medium loam.

Experimental materials: *Platycodon grandiflorum*: in mid-April, the seeds of *Platycodon grandiflorum*, which were full of maturity, were seeded after germination in early May, the line spacing was 20 cm × 20 cm, and the routine field management measures were adopted. *North Atractylodes*: was collected from the Dabie Mountains in Hubei Province, which was identified by the Institute of traditional Chinese medicine of Hubei province. At the end of the March, *Carthamustinctoriosa*, *Mentahaplocalyx* were sowed in the experimental base, soil preparation, fertilizing, weeding, ridging and field management were carried out according to agronomic measures of common origin.

Methods

Determination of photosynthetic rate of leaves

From each cultivated plant population, a number of leaves were selected to accept the natural solar radiation. The gas exchange measurement (Nie et al., 2014) was carried out by a portable optical gas analysis system (LI-6400, Li-CorInc, Lincoln NE, USA). The control of the environmental factors in the leaf chamber was 20 °C, the concentration of the gas source CO_2 was $350 \mu mol CO_2 mol^{-1}$, and the light flux density (PFD) was $1000 \mu mol photo ns^{-1} m^{-2} s^{-1}$. The experiment was carried out on all sunny days and repeated 5~6 times.

The calculation of the quantum efficiency (AQY) of the leaves shows a linear regression of the net photosynthetic rate (P_n) between $0 \sim 200 \mu mol photo ns^{-1} m^{-2} s^{-1}$ in the response curve to PFD, and the regression equation is obtained.

$$P_n = R_d + AQY \times PFD \quad (\text{Eq.1})$$

when $P_n = 0$, PFD is the optical compensation point Φ_i for photosynthesis. Where, R_d is the rate of dark respiration.

The calculation of the carboxylation efficiency of the leaves was linear regression on the $0 \sim 200 \mu\text{mol CO}_2 \text{ mol}^{-1}$ point in the C_i curve of the intercellular CO_2 concentration by P_n , and the regression equation was obtained.

$$P_n = R_p + CE \times C_i \quad (\text{Eq.2})$$

when $P_n = 0$, C_i is the CO_2 compensation point Γ of photosynthesis. R_p is the rate of respiration in the light, and CE is the carboxylation efficiency. As dark breathing is very small, the rate of CO_2 release can be approximated as the rate of photorespiration.

Determination of chlorophyll content

The leaves of the mature leaves with the diameter of 0.7 cm were collected from 30 mature leaves. The leaves were divided into 3 groups. They were immersed in the extract of 10 ml 80% acetone. The sample bottle was tightly sealed, and it was colorless under low temperature. When the concentration of leaf green was detected, the concentration of chlorophyll was obtained by detecting the light intensity of the fluorescence (Ribeiro-Filho et al., 2014). Portable optical gas analysis system is designed based on the principle that different gases have selective absorption of light. It is often used to detect carbon monoxide, carbon dioxide and other gases. The formula for calculating the fluorescence intensity is:

$$F = kQI_0(1 - e^{-\varepsilon cb}) \quad (\text{Eq.3})$$

in *Equation 3*: k is a constant; Q is the fluorescence efficiency; I_0 is the laser light intensity; c is the concentration of chlorophyll a; b is the light path for the test sample; ε is the molar absorbing coefficient.

In the way of logarithmic calculation, the formula is arranged and the logarithm is obtained.

$$C = A - B \log(D - F) \quad (\text{Eq.4})$$

where $A = \frac{2.3}{\varepsilon b} \log kQI_0$, $B = \frac{2.3}{\varepsilon b}$, $D = kQI_0$.

A , B , D in *Equation 4* are constant. When the sample of the fluorescence is determined, ε , k , Q , and I_0 are constant. Therefore, by measuring the fluorescence intensity of chlorophyll a emitted from cells, it can determine the concentration chlorophyll a and the fluorescence intensity of chlorophyll b to determine the concentration of chlorophyll b.

Determination of carotenoid content

The mixture of ethanol: acetone as 1:1 was used to extract 24 h in the dark, then be measured by UV-2001 ultraviolet spectrophotometer. And then the carotenoid content was calculated according to *Equation 5*.

$$C_x = (1000D_{470} - 2.05C_a - 114.8C_b) / 245 \quad (\text{Eq.5})$$

D_{470} is the absorbance at the wavelength of 470 nm. Pigment content = (pigment concentration × extract volume) / fresh weight of leaves.

Response of net photosynthetic rate to photosynthetically active radiation ($P_n - PAR$)

Using the red and blue light source, the light quantum flux density (PFD) in the Li-6400 portable photosynthetic system were 0, 400, 800, 1200, 1600, 2000, 2400 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively. The temperature was 27 °C and the concentration of CO_2 is set by CO_2 injection system to 390 $\mu\text{mol} \cdot \text{mol}^{-1}$, which was actually $390 \pm 10 \mu\text{mol} \cdot \text{mol}^{-1}$. The response of the net photosynthetic rate to the photosynthetic effective radiation was measured by the non-right angled hyperbolic model.

$$P_n = \frac{aI = P_{\max} - \sqrt{(aI + P_{\max})^2 - 4akIP_{\max}}}{2k} - R_d \quad (\text{Eq.6})$$

According to the light response equation, the simulated photosynthetic response curve was estimated, the light compensation point (LCP) and the optical saturation point (LSP) were calculated. That is to say, when the P_n reaches a certain level of P_{\max} , the I value was obtained, and the light saturation point was estimated (Shen et al., 2016). The value of I is used to estimate optical compensation point with P_n at 0. In Equation 6, P_n is the net photosynthetic rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), a is the use efficiency of optical quantum, I is the light flux density ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), P_{\max} is the maximum total photosynthetic rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), R_d is dark respiration efficiency ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and k reflects the degree of curvature of the photosynthesis curve.

Determination of photosynthesis and ecophysiological factors

Li-6400 portable photosynthesis measurement system produced by LI-COR Company was used. The net photosynthetic rate (P_n) of plant leaves was measured under natural condition. The time interval was 8:00–18:00, every 2 h was measured once, five values were recorded each time, and the average value was recorded. Li-6400 portable photosynthesis measurement system also recorded physiological and ecological factors such as temperature (T_a), relative humidity (RH), photosynthetically active radiation (PAR), stomatal conductance (G_s) and transpiration rate E, etc. (Ding et al., 2015).

Water use efficiency

Water use efficiency (WUE) = net photosynthesis rate * 44 / transpiration rate * 18, unit: $\text{mgCO}_2 / \text{gH}_2\text{O}$.

Results

The Figure 5 is the leaves' photosynthetic rate comparison chart of four species of medicinal plants during the whole growth period. The Figure 6 is a daily change map of

the environment factor. The *Figure 7* is the effect of net photosynthetic rate on photosynthetically active radiation.

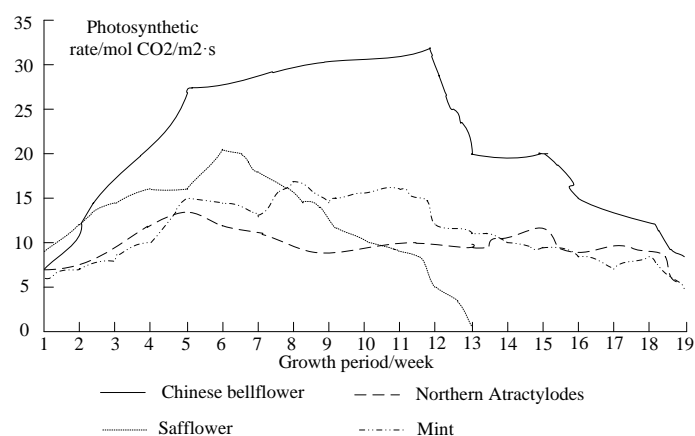


Figure 5. Contrast diagram of photosynthetic rate of leaves at full growth stage

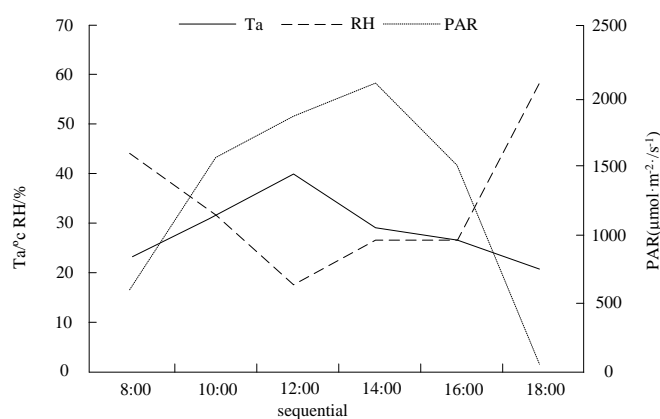


Figure 6. Diurnal variation of environmental factors

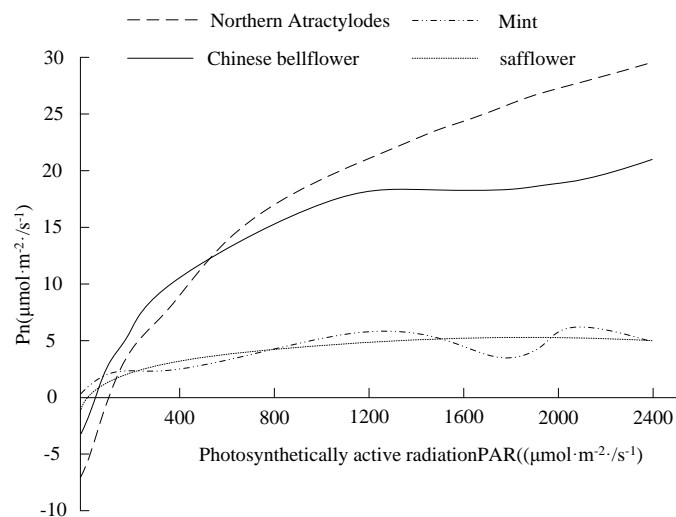


Figure 7. Effect of net photosynthetic rate on photosynthetically active radiation

Table 1 is the photosynthetic parameters of four medicinal plants. Table 2 is the content of photosynthetic pigments in four medicinal plants. Table 3 is the path analysis between the main factors affecting the net photosynthetic rate.

Table 1. Photosynthetic parameters of 4 medicinal plants

Medicinal plant	LCP ($\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$)	LSP ($\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$)	Pmax ($\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$)
<i>Platycodon grandiflorum</i>	45 ± 6.03 b	1052 ± 113.16 b	21.8 ± 2.02 b
<i>North Atractylodes</i>	106 ± 10.97 a	1558 ± 162.97 a	29.7 ± 2.86 a
<i>Carthamustinctorios</i>	38 ± 6.56 b	307 ± 42.67 c	5.8 ± 0.76 c
<i>Menthahaplocalyx</i>	7 ± 1.53 c	261 ± 12.17 c	5.0 ± 0.53 c

The difference between the different letters in the same column is significant ($p < 0.05$), the same below

Table 2. Content of photosynthetic pigments in 4 medicinal plants

Medicinal plant	Chlorophyll ($\text{mg}\cdot\text{g}^{-1}$)	Carotenoid ($\text{mg}\cdot\text{g}^{-1}$)	Chlorophyll a/b
<i>Platycodon grandiflorum</i>	1.769 ± 0.021 2 b	0.342 ± 0.016 4 b	2.880 ± 0.007 0 b
<i>North Atractylodes</i>	1.085 ± 0.032 1 c	0.204 ± 0.015 1 c	3.189 ± 0.402 0 a
<i>Carthamustinctorios</i>	1.919 ± 0.061 7 a	0.417 ± 0.020 4 a	2.861 ± 0.086 3 b
<i>Menthahaplocalyx</i>	1.701 ± 0.102 1 b	0.396 ± 0.017 8 a	2.471 ± 0.073 2 c

Table 3. Path analysis between the main factors affecting the net photosynthetic rate

Medicinal plant	Influence factor	Direct action	Indirect effect of Ta	Indirect effect of RH	Indirect effect of PAR	Indirect effect of Gs	Total effect	Decision coefficient
<i>Platycodon grandiflorum</i>	Ta	-0.247 2		-0.001 6	0.225 2	0.020 1	0.178 5	-0.149 380
	RH	0.001 7	0.220 8		-0.230 7	-0.271 1	-0.279 3	-0.000 950
	PAR	0.368 7	-0.150 9	-0.001 1		0.436 0	0.652 7**	0.345 357
	Gs	0.810 3	-0.061 6	-0.000 6	0.198 4		0.946 5**	0.877 275
<i>North Atractylodes</i>	Ta	-0.000 8		-0.108 1	0.244 4	0.104 9	0.240 4	-0.000 390
	RH	0.121 1	0.000 8		-0.232 5	-0.200 4	-0.311 0	-0.090 010
	PAR	0.333 9	-0.000 6	-0.084 3		0.502 5	0.751 5**	0.390 402
	Gs	0.793 8	-0.000 1	-0.030 6	0.211 4		0.974 5**	0.917 012
<i>Carthamustinctorios</i>	Ta	-0.064 2		-0.134 4	0.092 8	-0.130 8	-0.236 6	0.026 247
	RH	0.154 7	0.055 7		-0.061 9	-0.104 2	0.044 3	-0.010 180
	PAR	0.142 5	-0.041 8	-0.067 1		0.255 8	0.289 4	0.062 163
	Gs	0.942 9	0.008 9	-0.017 1	0.38 7		0.973 4**	0.946 522
<i>Menthahaplocalyx</i>	Ta	-2.669 7		1.328 0	0.883 1	0.092 6	-0.366 0	-5.173 310
	RH	-1.504 3	2.357 0		-0.624 9	-0.031 0	0.196 8	-2.854 550
	PAR	1.371 3	-1.719 4	0.685 5		-0.036 5	0.300 9	-1.054 840
	Gs	-0.235 1	1.052 0	-0.198 2	0.212 8		0.831 5**	-0.446 230

Figure 8 is the diurnal variation of the stomatal conductance and net photosynthetic rate. Figure 9 is a seasonal change in net photosynthetic rate.

Table 4 is the changes of transpiration rate (unit: mg/g FW) of *Platycodon grandiflorum* and *North Atractylodes* in the whole growth period. Table 5 is the changes of transpiration rate (unit: mg/g FW) of *Carthamustinctorios* and *Menthahaplocalyx* in the whole growth period. Table 6 is the water use efficiency of *Platycodon*

grandiflorum and *North Atractylodes* in the whole growth period (unit $mgCO_2 / gH_2O$). Table 7 is the water use efficiency of *Carthamustinctoriosis* and *Mentahaplocalyx* in the whole growth period (unit $mgCO_2 / gH_2O$).

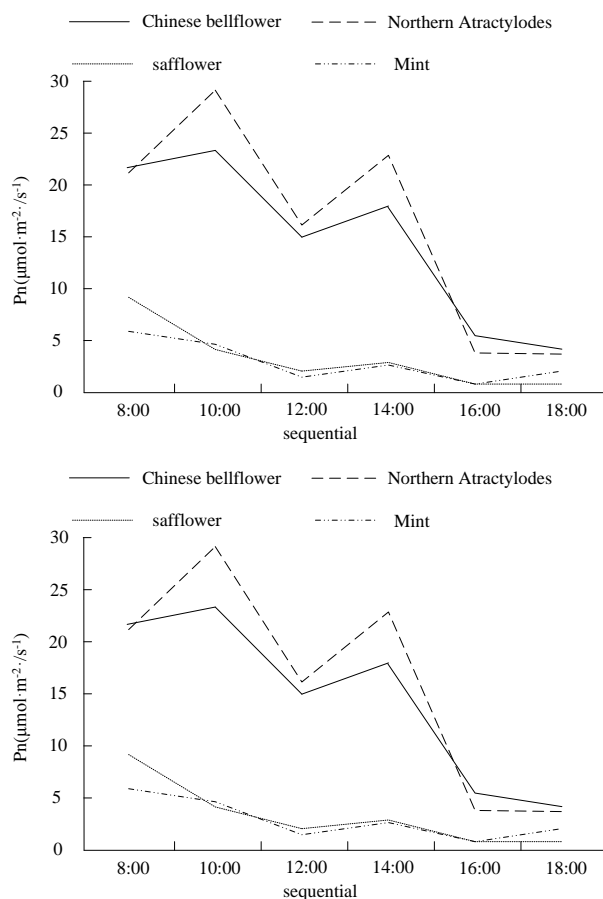


Figure 8. Diurnal variation of stomatal conductance and net photosynthetic rate

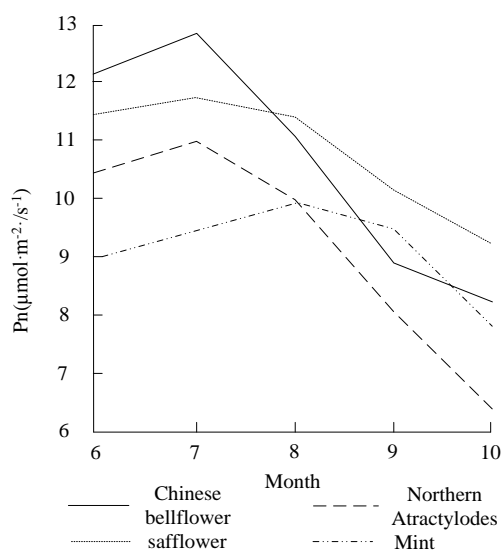


Figure 9. Seasonal variation of net photosynthetic rate

Table 4. Change of transpiration rate of *Platycodon grandiflorum* and *North Atractylodes* during the whole growth period

<i>Platycodon grandiflorum</i>			<i>North Atractylodes</i>		
Period of birth	Date	Transpiration rate/g•m ⁻² •h ⁻¹	Period of birth	Date	Transpiration rate/g•m ⁻² •h ⁻¹
Seedling stage	05-10	1.50	Seedling stage	05-10	2.06
	05-24	1.66		05-17	2.04
	06-07	2.27		05-24	2.86
Rosette stage	06-14	4.02	Florescence	06-10	3.81
	06-22	3.42		06-22	3.06
	07-07	3.09		07-01	2.41
Florescence	07-25	2.43	Fruit	07-13	2.33
	08-05	2.54		07-19	2.33
	08-16	1.81		07-25	2.29
Fruit	08-31	1.52	Bulking period of tuber	08-05	2.78
	09-10	1.27		08-16	2.90
	09-26	1.28		08-31	2.74
			Tuber weight gain period	09-10	2.04
				09-25	1.63

Table 5. The change of transpiration rate in the whole growth period of *Carthamustinctorios* and *Menthahaplocalyx*

<i>Carthamustinctorios</i>			<i>Menthahaplocalyx</i>		
Period of birth	Date	Transpiration rate/g•m ⁻² •h ⁻¹	Period of birth	Date	Transpiration rate/g•m ⁻² •h ⁻¹
Rosette stage	5.10	1.52	Seedling stage	5.10	1.43
	5.17	2.66		5.17	2.12
	5.24	2.37		5.24	2.65
Branching period	6.1	2.97	Branching period	6.7	2.81
	6.7	3.13		6.14	2.40
	6.14	3.43		6.22	2.99
Florescence	6.22	2.75	Florescence	7.7	2.91
	7.1	2.32		7.19	2.90
	7.13	1.59		7.25	2.44
Fruit	7.19	1.63	Fruit	8.5	2.39
	7.25	0.20		8.16	2.09
				8.31	1.92

Table 6. Water use efficiency of *Platycodon grandiflorum* and *North Atractylodes* at full growth stage

<i>Platycodon grandiflorum</i>			<i>North Atractylodes</i>		
Period of birth	Date	WUE (g/kg)	Period of birth	Date	WUE (g/kg)
Seedling stage	5.10	11.37	Seedling stage	5.10	2.06
	5.17	10.63		5.17	2.04
	5.24	8.93		5.24	2.86
Rosette stage	6.1	11.35	Florescence	6.11	3.81
	6.7	16.82		6.22	3.06
	6.14	12.49		7.1	2.41
Florescence	7.20	13.38	Fruit	7.13	2.33
	8.15	12.96		7.19	2.33
	9.5	12.91		7.25	2.29
Fruit	9.10	10.56	Bulking period of tuber	8.5	2.78
	9.17	9.81		8.16	2.90
	9.25	8.02		8.31	2.74
			Tuber weight gain period	9.10	2.04
				9.25	1.63

Table 7. Water use efficiency of the full growth period of *Carthamustinctorios* and *Mentahaplocalyx*

<i>Carthamustinctorios</i>			<i>Mentahaplocalyx</i>		
Period of birth	Date	WUE	Period of birth	Date	WUE
Rosette stage	5.10	12.39	Seedling stage	5.10	11.29
	5.17	11.66		5.17	10.02
	5.24	14.84		5.24	8.92
Branching period	6.1	12.88	Branching period	6.07	11.54
	6.7	12.78		6.14	15.96
	6.14	13.79		6.22	11.90
Florescence	6.22	15.89	Florescence	7.7	13.03
	7.1	16.45		7.19	12.86
	7.13	16.92		7.25	12.98
Fruit	7.19	11.56	Fruit	8.5	11.90
	7.25	7.57		8.16	10.49
	7.31	5.42		8.31	8.95

Data analysis

The following results are derived from the experiments in the previous section.

Comparison of photosynthetic rate in whole growth period

From *Figure 5*, we can see that in the whole growth period, the net photosynthetic rate of medicinal plants propagated by seeds showed a single peak curve: that is, the seedling stage is low, and the net photosynthetic rate increases with the expansion of vegetative body. When it reached high in the period of flower and fruit, it was gradually reduced; while the change curve of net photosynthetic rate of the medical plants propagated by rhizome showed bimodal curve. This is because the physiological and biochemical characteristics of medicinal plants are also different. Therefore, there are some differences in net photosynthetic rate.

The seasonal variation of net photosynthetic rate

Figure 9 showed that plant leaves had matured in June, and the photosynthetic rate of the four medicinal plants began to rise, and the maximum of the growth season was reached about July. With the change of season, photosynthetic rate began to decrease. In October, photosynthetically active radiation and air temperature decreased, leaves began to senescence, physiological activity decreased, and photosynthetic rate decreased to the minimum value in growing season. In addition, four kinds of medicinal plant photosynthetic rate in the growing season was significantly higher than that at the end of the growing season, this was because the higher leaf physiological activity of plant growth season (Chen et al., 2015). But with the leaf senescence and seasonal variation of environmental factors, the physiological activity of leaves decreased, the efficiency of photosynthetic apparatus declined, and the ability of assimilating CO₂ decreased, which showed that the photosynthetic rate of all plants decreased.

Diurnal changes of environmental factors

The environmental factors that affect plant photosynthesis include temperature, moisture, photosynthetic effective radiation, etc. The changes of temperature (T_a), relative humidity (RH) and photosynthetically active radiation (PAR) in the test day were shown in *Figure 6*.

In one day, the change of three environmental factors showed a single peak. PAR increased from $589 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to $1989 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in 8:00-14:00, and then dropped to a minimum at 18:00, only $91 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. T_a was mainly affected by PAR ($r = 0.899^{**}$), the largest was at 12:00, as 40.9°C , and to 22.3°C at 18:00. RH and PAR was significantly negatively correlated with T_a ($p < 0.01$). The correlation coefficient r_1 and r_2 were -0.945^{**} , -0.941^{**} , respectively.

Diurnal variation of stomatal conductance (G_s) and net photosynthetic rate (P_n)

It can be clearly seen from *Figure 8* that the changes of G_s and P_n of *Platycodon grandiflorum* and *North Atractylodes* were typical bimodal curve in one day, and the first peak > the second peak. This is due to the low light intensity and low temperature in the morning. At about 10:00, it reached the first peak, at this time PAR was $1161 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and T_a was 32°C . At about 12:00, *Platycodon grandiflorum* and *North*

Atractylodes were in the “noon break” state of photosynthesis, G_s decreased, and P_n decreased. At about 14:00, G_s and P_n reached second peak. G_s and P_n of *Carthamustinctorios* and *Mentahaplocalyx* were the largest at 8:00, and they were at a lower level since then, and there was no obvious “noon break” phenomenon.

The daily change of net photosynthetic rate of *Platycodon* *Platycodon* and *North Atractylodes* was bimodal curve, and the “noon break” phenomenon of photosynthesis appeared. This is a kind of beneficial ecological adaptation and self-regulation mechanism for the hot summer in the plant.

The relationship between the diurnal variation of net photosynthetic rate (P_n) and ecophysiological factors

The path analysis results of P_n and eco physiological factors (Table 3) showed that P_n and PAR of *Platycodon grandiflorum* and *North Atractylodes* was significantly positively correlated with G_s , but without correlation of RH and T_a . P_n of *Carthamustinctorios* and *Mentahaplocalyx* were positively correlated with G_s , not related to RH and PAR, but negatively correlated with T_a . According to the physiological and ecological factors, the decision coefficient P_n of the four medicinal plants in a day showed that the determinants that affected the diurnal variation of *Platycodon grandiflorum* and *North Atractylodes* were PAR and G_s , and PAR was the main determining factor. Limiting factors were T_a and RH, where T_a was a major limiting factor for the diurnal variation of *Carthamustinctorios* P_n , and the limiting effect of T_a and RH on *North Atractylodes* P_n was small. G_s was the main determinants of the diurnal variation of *Carthamustinctorios*, T_a and PAR also had certain promoting effect on P_n , but the effect was very small; RH was a limiting factor of diurnal variation of *Carthamustinctorios* P_n . T_a , RH, PAR and G_s were the limiting factors of the diurnal variation of *Mentahaplocalyx* P_n , and T_a of them were the most important limiting factor.

Comparison of photosynthetic parameters

The light compensation point (LCP), the light saturation point (LSP) and the maximum net photosynthetic rate can directly reflect the tolerance and photosynthetic capacity of plants. The low LCP of plants means that plants have strong ability to use weak light. The low LSP showed that the photosynthetic rate of plants increased rapidly with the increase of light quantum density, and quickly reached the maximum efficiency. Therefore, lower LCP and LSP enable plants to maximize photosynthetic capacity under low light density and maximize their photosynthesis under light limitation, thereby enhancing the accumulation of organic matter and meeting their energy needs for survival and growth.

It can be seen from the light response of P_n (Fig. 7) that the order of respiration rate of four medicinal plants were: *Mentahaplocalyx* < *Carthamustinctorios* < *Platycodon grandiflorum* < *North Atractylodes*. In the PAR range of $0 \sim 100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the P_n and PAR of four kinds of medicinal plants was closed to the linear relationship. The P_n of *Mentahaplocalyx* in the range was the maximum and that of *North Atractylodes* was the minimum. P_n of *Carthamustinctorios* and *Mentahaplocalyx* increased slowly in

that PAR was $100 \sim 300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and reached saturation, and then tended to be stable after $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. P_n of *Platycodon grandiflorum* and *North Atractylodes* increased rapidly in that PAR was $100 \sim 1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and closed to saturation in about $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $1600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The light compensation curve (LCP), light saturation point (LSP) and maximum net photosynthetic rate of the four medicinal plants were estimated according to the fitting equation of light response curve, shown in Table 3.

The results showed that the LCP, LSP and the maximum net photosynthetic rate of the *North Atractylodes* were significantly higher than those of the other 3 medicinal plants, indicating that they had strong ability to use intense light and high photosynthetic capacity under intense light, and $106 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of LCP showed their shade tolerance. The LCP of *Carthamustinctorios* and *Menthahaplocalyx* was lower than that of the *North Atractylodes*, indicating that they had high ability to use weak light and strong shade tolerance. The LCP of *Platycodon grandiflorum* was close to *Carthamustinctorios*, while LSP was higher, and has a higher maximum net photosynthetic rate, indicating that its photosynthesis was effective and its adaptability to light was the largest. The maximum net photosynthetic rate of *Carthamustinctorios* and *Menthahaplocalyx* was significantly lower than that of *Platycodon grandiflorum* and *North Atractylodes*, while *Platycodon grandiflorum* was lower than that of *North Atractylodes*.

Water use efficiency in the production of photosynthetic substances in medicinal plants

It can be seen from Tables 6 and 7 that in the 4 medicinal plants, except the *North Atractylodes*, the remaining plants have a common characteristic in the water use efficiency, that is, the single peak curve changes in the whole growth period.

Differences between species in water use efficiency: Although the change of water use efficiency during the growth period is a single peak curve, for different plant species, water use efficiency has the characteristics of species, mainly in the following areas:

Firstly, the comparison of the whole growth period, *Platycodon Grandiflorum* has the highest water use efficiency of $15.62 \sim 23.22 \text{ mgCO}_2 / \text{gH}_2\text{O}$, followed by *Carthamustinctorios*, *Menthahaplocalyx*, and the lowest is the *North Atractylodes*.

Secondly, the period of highest water use efficiency has interspecific difference: *Platycodon grandiflorum*, *Carthamustinctorios* and *Menthahaplocalyx* propagated by seeds is at flowering stage, while the *North Atractylodes* propagated by vegetative is in accumulation stage.

Thirdly, the water use efficiency of *Platycodon grandiflorum* is relatively stable during the whole growth period, that is, there is little difference in the growth period. For example, the water use efficiency of *Platycodon grandiflorum* at different growth stages was 7.21%-11.63% compared with the water use efficiency of the whole growth period, while the differences in the three other medicinal plants was more than 50%.

Change of transpiration rate of medicinal plants in the whole growth period

Transpiration is the process of loss of water from the surface of the living plant (mainly leaves) in the state of water vapor into the atmosphere. It is different from the

evaporation process of physics. Transpiration is not only influenced by environmental conditions, but also regulated and controlled by the plant itself, so it is a complex physiological process.

Transpiration is of great importance to plants, providing the main motive force for the plant to absorb and transport water, and also to lose the water. Photosynthesis is a way for plants to produce organic matter by means of light energy. It is often related to the importance of plant growth and crop yield.

The results of *Tables 4* and *5* showed that the change of transpiration rate of different medicinal plants was similar to that of their net photosynthetic rate. The total change trend of transpiration rate in the whole growth period of the medicinal plants propagated by seeds showed a single peak curve: that is, the seedling stage was low, and transpiration rate gradually increased with the continuous expansion of the vegetative body. After reaching the highest level, it gradually reduced to the lowest in the late growth stage, and the transpiration rate of *Platycodon grandiflorum* varied greatly, While the *North Atractylodes*, which propagated with rhizomes and tubers, showed the bimodal curve.

There are some differences between the transpiration rates of medicinal plants: the *Platycodon grandiflorum* has the maximum transpiration rate, especially in the branching stage and the early flowering stage, between $2.43 \sim 4.02 \text{ m mol H}_2\text{O/m}^2/\text{s}$, followed by *Carthamustinctorios* and *Mentahaplocalyx*, and the lowest is *Atractylodis sinensis*. The changes of the whole growth period of the *Atractylodis sinensis* is more stable.

Discussion

Photosynthesis is the process of green plant uses visible light energy to synthesize the carbon dioxide and hydration to become carbohydrate (usually glucose) through the chloroplast, and release oxygen (Zhu et al., 2016). The reaction equation of photosynthesis is: $6\text{CO}_2 + 12\text{H}_2\text{O} \xrightarrow[\text{Chloroplast}]{\text{Luminous energy}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 6\text{H}_2\text{O}$. Net photosynthetic rate is closely related to its own factors, such as chlorophyll content, leaf thickness and leaf maturity. It is also affected by light intensity, air temperature, air relative humidity and soil water content (Schwingamer et al., 2015).

Analysis of photosynthetic rate during the whole growth period

Among the four studied medicinal plants, *Platycodon grandiflorum* should be C4 plant and the other three species of medicinal plants are C3 plants. In each growth period, the net photosynthetic rate of *Platycodon grandiflorum* was significantly higher than that of *North Atractylodes*, *Carthamustinctorios*, *Mentahaplocalyx* cultivated in the same medicine Park. This is due to the C4 pathway of *Platycodon grandiflorum*, which has a fixed CO_2 , and the source of energy is continuously supplied and fully utilized by CO_2 , so it has a higher net photosynthetic rate (Schwingamer et al., 2015).

The results showed that the photosynthetic rate of *Carthamustinctorios* and *Mentahaplocalyx* was slightly lower than that of *North Atractylodes* in the early stage of growth, while in the middle growth stage, it was significantly higher than that of *North Atractylodes*. The reason may be related to the difference of propagation mode (Peng et al., 2015): rhizome was used as propagating material for *North Atractylodes*, and soon entered fast growing stage, which showed strong photosynthetic ability. In

addition, the time when the *North Atractylodes* entered the flowering and fruiting stage was earlier than that of *Carthamustinctorios* and *Menthahaplocalyx*, and there was a strong physiological bank.

In the middle growth period, the rapid growth of *Carthamustinctorios* and *Menthahaplocalyx* needs large amounts of organic material for the vegetative growth (Almeida et al., 2014), and although *Atractylodes sinensis* at this time also began to rapid growth of vegetative, because the strain was smaller and the consumption of organic substances were also relatively less. The net photosynthetic rate of the former is not high.

The difference in the medicinal parts was different: rhizome was the storage location of *North Atractylodes*, and it had swelling and nutrient accumulation in the late growth stage (Kjaer et al., 2014), thus showing another peak of net photosynthetic rate.

The seasonal variation of net photosynthetic rate is discussed

In June to July, the ability of *Platycodon grandiflorum* root, *Carthamustinctorios* and *North Atractylodes* to use high temperature and high light intensity was obviously better than that of *Menthahaplocalyx*; In August and September, the photosynthetic capacity of *Menthahaplocalyx* increased slightly, indicating that *Menthahaplocalyx* could make use of relatively low light and temperature conditions more effectively. The photosynthetic rate of the *North Atractylodes* was the lowest in September and October, indicating that its ability to use relatively low light and temperature conditions was the lowest in four medicinal plants. The results showed that in the growing season, the order of average photosynthetic rate of four species of medicinal plants was: *Platycodon grandiflorum* > *Carthamustinctorios* > *Menthahaplocalyx* > *North Atractylodes*.

The diurnal variations of stomatal conductance and net photosynthetic rate are discussed

The plant self-factors that cause the net photosynthetic rate of plant leaves decreased at noon including stomatal and non-stomatal factors. At noon, in the field, the intense light, high temperature, low humidity and other environmental factors caused by plant stomatal closures, CO_2 was blocked to enter into the leaves and photosynthesis decreased, while non-stomatal factor was because the temperature was too high, resulting in a decline in net photosynthesis in mesophyll cells. In addition, under high light intensity for a long time, light inhibition and photorespiration can also lead to a decrease in net photosynthetic rate at noon. The response of P_n from *Platycodon grandiflorum* and *North Atractylodes* to PAR showed that *Platycodon grandiflorum* and *North Atractylodes* still had high P_n (above 19.5 and 26, $26.6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) under intense light (above $1800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), so photoinhibition and photorespiration were not the factors limiting P_n at this time. At 12:00, RH dropped to the lowest point of the day, and G_s was also lower than 10:00 and 14:00, so the stomatal factor was one of the reasons for the low net photosynthetic rate at this time. In addition, T_a reached 40.9°C at this time. Previous studies found that the optimum activation temperature for the key enzyme of Rubisco in photosynthesis was at $25\sim 30^\circ\text{C}$, its activity directly affects the photosynthetic rate. When the leaf temperature is too high, it will affect the activities of some related enzymes in the body, which is not conducive to the photosynthesis.

Therefore, the decline of P_n in the two plants is also caused by the decrease of the non-stomatal factors, the activity of the carboxycarboxylase of mesophyll cells.

The net photosynthetic rate of *Carthamustinctorios* and *Mentahaplocalyx* had a diurnal variation. The maximum net photosynthetic rate of the two plants was $9.1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and $5.8 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ respectively. After that, the net photosynthetic rate declined rapidly, which was similar to the results of the diurnal variation of the photosynthesis by Zhang Jingguang, et al. In the response of the P_n of the two plants to the PAR, the P_n decreased at $\text{PAR} > 1400 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, indicating that the photoinhibition might have occurred under the intense light. The correlation analysis between diurnal variation of photosynthesis and physiological and ecological factors showed that G_s is the main influence factor of *Carthamustinctorios* P_n , while G_s of the *Carthamustinctorios* is low in a day, so stomatal limitation is a cause of low P_n in the daytime. It is believed that high temperature and intense light cause the potential activity of the active center and the primary light conversion efficiency decreasing, which hindered the photosynthetic electron transport. It is the limiting factor of photosynthesis (Zhang et al., 2015).

Content of photosynthetic pigment

The pigment that absorbs light in photosynthesis is called photosynthetic pigment, and there are two main types in higher plants: chlorophyll (including chlorophyll a, b) and carotenoids. The color of the leaves is mainly a comprehensive representation of the proportion of green chlorophyll and yellow carotenoid. The chlorophyll a/b of the leaves of the common sun plants is about 3:1, and the chlorophyll a/b of the shade plants is 2:3:1. The relative increase of chlorophyll b content may make more effective use of the blue violet light in the leaching light. So the chlorophyll b has the name of the chromophore system. The chlorophyll content of plants depends not only on site conditions, but also on the characteristics of plant species, and determines the ability of plants to absorb and convert light energy (Li et al., 2016).

The content of chlorophyll is also related to the resistance of plants to the shade. The chlorophyll content of *Platycodon grandiflorum* and *Mentahaplocalyx* had not significantly different, but the LSP and the maximum net photosynthetic rate of *Platycodon grandiflorum* were significantly higher than that of *Mentahaplocalyx*. The chlorophyll content of the *North Atractylodes* was significantly lower than that of *Platycodon grandiflorum*, but LSP was significantly higher than that of *Platycodon grandiflorum*, and the maximum net photosynthetic rate of them had no significant difference. The results showed that the content of chlorophyll was not directly related to LSP and the maximum net photosynthetic rate. The LCP of *Platycodon grandiflorum*, *Carthamustinctorios* and *Mentahaplocalyx* were lower than that of the *North Atractylodes*, so does the chlorophyll a/b value, but the content of chlorophyll and carotenoid was higher than that of the *North Atractylodes*, this may be because the plants with high chlorophyll content have weak absorption ability of light, it has better resistance to shade. In addition, it was found that low chlorophyll a/b value increased the absorption of far red light. Therefore, plants with low chlorophyll a/b value also had higher photosynthetic activity under low intensity of light.

In the experiment, the chlorophyll content of *Carthamustinctorios* was the highest in the four medicinal plants, followed by *Platycodon grandiflorum* and

Menthahaplocalyx, and that of *North Atractylodes* was the lowest. However, the content of chlorophyll a/b of *North Atractylodes*'s is the highest, followed by *Platycodon grandiflorum* and *Carthamustinctorious*, and that of *Menthahaplocalyx* leaves is the lowest. *Carthamustinctorious* and *Menthahaplocalyx* in four medicinal plants have the highest carotenoid content.

Conclusions

With the continuous progress of the social development and the accelerated process of industrialization, agriculture and green land area is decreased. The study of Photosynthetic Physiology plays a very important role in the selection of germplasm resources, the rational allocation of planting environment and the increase of yield in medicinal plants (Niakan et al., 2015). According to the first two chapters, we can further understand their photosynthetic and physiological characteristics by studying and discussing four kinds of medicinal plants of *Platycodon grandiflorum*, *Atractylodis sinensis*, *Carthamustinctorious* and *Menthahaplocalyx*.

The *Atractylodis sinensis* has high utilization rate of intense light, but it belongs to the light plant, and has poor shade tolerance. Thus, it should be cultivated in the environment of direct sunlight enough to increase the yield. *Carthamustinctorious* and *Menthahaplocalyx* are shade loving plants, but they are not resistant to intense light. The cultivation environment outside the forest is easy to inhibit their photosynthesis. Therefore, the intercropping way of forest and medical plants can be adopted. *Platycodon grandiflorum* has great adaptability to light intensity. It can not only efficiently use intense light but also have a characteristic of certain shade tolerance. If it wants to increase photosynthetic rate and increase yield, it is recommended to cultivate it in an intense light environment.

In the future research stage, the ecological characteristics of the same species of medicinal plants in different growing areas will be studied in depth, so as to provide more theoretical support for the development and utilization of medicinal plants.

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