# SEASONAL VARIATION CHARACTERISTICS OF CHLOROPHYLL AND SPECTRUM IN LEAVES OF *Populus euphratica* UNDER WATER STRESS**

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*Chlorophyll content is an important index for monitoring the health status of vegetation growth. Revealing the variation characteristics of the chlorophyll content in leaves of Populus euphratica plays an important role in evaluating the health and ecological conservation of Populus euphratica. In this study, leaves of healthy Populus euphratica and water-stressed Populus euphratica were collected from May to October, and the spectral reflectance was obtained at 390–1100 nm. At the same time, the chlorophyll content of Populus euphratica leaves was analyzed. The results indicated that from May to October, the chlorophyll content showed a trend of "two peaks and two valleys," which decreased first, then increased and then decreased. In general, the chlorophyll content of healthy Populus euphratica leaves was higher than that of water-stressed Populus euphratica leaves. The ratio of chlorophyll a to chlorophyll b was maintained between 3.0 and 3.4 from May to August, while there was a significant increase in September and October. The ratio of chlorophyll a to chlorophyll b in healthy Populus euphratica leaves was significantly lower than that in water-stressed Populus euphratica leaves from June to September. The spectral curves of healthy Populus euphratica leaves were relatively consistent from May to August, and the curves in the near-infrared band were also concentrated. For the water-stressed Populus euphratica leaves, the curve in the near-infrared band was more dispersed. There were two absorption valleys at wavelengths of 500 and 675 nm, and some of the absorption characteristic parameters of the two absorption valleys had a significant correlation with the chlorophyll content. A partial least squares regression model constructed by these absorption parameters roughly estimated the chlorophyll content of the water-stressed Populus euphratica leaves.*

***Keywords***: *variation characteristics, Populus euphratica, chlorophyll content, hyperspectrum.*

# ХАРАКТЕРИСТИКИ СЕЗОННОЙ ИЗМЕНЧИВОСТИ ХЛОРОФИЛЛА И СПЕКТРА ЛИСТЬЕВ *Populus euphratica* ПРИ ВОДНОМ СТРЕССЕ

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*УДК 535.34:547.979.7*

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** Full text is published in JAS V. 88, No. 3 (http://springer.com/journal/10812) and in electronic version of ZhPS V. 88, No. 3 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

*(Поступила 26 декабря 2019)*

*Для мониторинга состояния роста растений исследованы характеристики основных компонентов хлорофилла в листьях тополя Populus euphratica, здоровых и испытывающих водный стресс, собранных с мая по октябрь. Коэффициент отражения получен в диапазоне 390–1100 нм. Проанализировано содержание хлорофилла в листьях Populus euphratica. С мая по октябрь содержание хлорофилла демонстрирует тенденцию "два пика и две впадины": уменьшение, увеличение и снова уменьшение. В целом содержание хлорофилла в здоровых листьях выше, чем в листьях, испытывающих водный стресс. Отношение хлорофилла a к хлорофиллу b (Хлa/Хлb) поддерживалось от 3.0 до 3.4 с мая по август, тогда как в сентябре и октябре значительно увеличивалось. Отношение Хлa/Хлb в здоровых листьях значительно ниже, чем в испытывающих водный стресс, с июня по сентябрь. Спектральные кривые относительно постоянны для здоровых листьев с мая по август, а в ближнем ИК-диапазоне еще и сконцентрированы. Для листьев, подверженных водному стрессу, кривые в ближнем ИК-диапазоне более рассредоточены. Наблюдаются два минимума поглощения на длинах волн 500 и 675 нм, некоторые из характеристических параметров поглощения этих минимумов имеют значительную корреляцию с содержанием хлорофилла. Модель регрессии методом частичных наименьших квадратов, построенная по параметрам поглощения, позволяет оценить содержание хлорофилла в листьях Populus euphratica, испытывающих водный стресс.*

***Ключевые слова:*** *вариационные характеристики, Populus euphratica, содержание хлорофилла, гиперспектр.*

**Introduction.** Chlorophyll is a photosynthetic pigment of plants. Understanding the changes in chlorophyll content with the changes in season has important theoretical and practical significance for revealing the photosynthetic physiological response of vegetation. *P. euphratica* (*Populus euphratica*) is distributed in more than 20 countries in Asia, Europe, and Africa and is one of the most widely distributed desert area taxa in Central Asia [1]. The most widely distributed and abundant areas in the world for this species are the oases of China, mainly in the Xinjiang Uygur Autonomous Region, Hetao Plain, the Alxa Plateau, the Qaidam Basin, and the Hexi Corridor [2]. *P. euphratica* is an ancient and endangered plant, and it is the only arbor that has established a population in the desert riparian ecosystem of arid areas and is also the dominant tree species that maintains the ecological balance in the desert-oasis ecotone [3]. Due to the deteriorating living environment, the protection of *P. euphratica* is extremely urgent [4]. Chlorophyll content is often associated with plant health [5]. Therefore, the chlorophyll change characteristics of *P. euphratica* leaves were studied to provide a theoretical basis for the ecological conservation of *P. euphratica*. At present, there is no research on the seasonal variation characteristics of chlorophyll in leaves of water-stressed *P. euphratica*. Most studies focus on the physiological and ecological processes of *P. euphratica* under stress. Zhao et al. [6] studied the variation characteristics of the physiological parameters of *P. euphratica* under salt stress and found that the net photosynthetic rate, intercellular $CO_2$ concentration, stomatal conductance, transpiration rate, and chlorophyll fluorescence value decreased with increasing salt concentrations, while proline increased with increasing salt concentrations. Keyimu et al. [7] estimated the water consumption of desert riparian forest in the lower reaches of the Tarim River by measuring the runoff of *P. euphratica*. Presently, there have been some studies on the spectral characteristics of *P. euphratica* leaves, mainly on the estimation of the pigment content in leaves. Studies on the chlorophyll content in leaves of *P. euphratica* have mainly focused on the estimation of the chlorophyll content using hyperspectral variables. Xu et al. [8] analyzed the correlation between the spectral reflectance of visible light and near-infrared band and chlorophyll content in leaves of *P. euphratica* and found that the reflectance at the two wavelengths of 645 and 769 nm could be used to estimate the chlorophyll content in leaves of *P. euphratica*. Wang et al. [9] used the hyperspectral index and vegetation index to estimate the chlorophyll content in the leaves of *P. euphratica*. In this study, the seasonal variation characteristics of the chlorophyll content and spectral parameters in healthy and water-stressed *P. euphratica* leaves were analyzed to provide a theoretical basis for ecological environment construction in arid areas.

**Experimental.** The Tarim River is located on the northern edge of the Tarim Basin in Xinjiang, China and is adjacent to the Taklimakan Desert. It is the longest inland river in China, with a total length of 1321 km. The Tarim River is formed by the confluence of three rivers, the Aksu River, the Yarkand River, and the Hotan River, which are fed by glacial meltwater, groundwater, and precipitation [10]. The desert riparian forest on both sides of the river basin serves as an ecological barrier between desert and oasis, and the forest is mainly

*P. euphratica*. It is the most important population of forest vegetation in the desert-oasis ecotone. The Tarim River basin experiences a relatively dry climate, strong evaporation, and low precipitation.

Our study area was located in the Awat County *P. euphratica* nature reserve (80°20′30″–80°24′00″E, 40°27′00″–40°30′00″N) of the upper reaches of the Tarim River, which is in the lower reaches of the Yarkand River. The region is one of the driest areas in China and belongs to the warm temperate arid desert climate, with an average annual rainfall of 50.4 mm, an average annual evaporation capacity of 1880.0 mm, annual average sunshine hours of 729.0 h, annual solar radiant energy of 604.57 kJ/cm$^2$, an annual average temperature of 10.4°C, a greater than or equal to 10°C accumulated temperature of 4138°C, an extreme maximum temperature of 39.4°C, an extreme minimum temperature of 25.0°C, and a frost-free period of approximately 205 days. The temperature is very hot during the day and night.

In the past 50 years, the exploitation and utilization of water and soil resources by humans has caused the decline of the groundwater level in the upper reaches of the Tarim River, aggravated the drought stress degree of the desert vegetation living environment, and resulted in the reduction of the area of desert riparian forest and the gradual disappearance of *P. euphratica* forest [11].

Two sampling areas were selected: one was the sampling area of healthy *P. euphratica*, which was approximately 500 m from the river channel, where the groundwater depth was less than 2 m, and the other was the sampling area of the water-stressed *P. euphratica*, which was approximately 4 km from the river channel. The groundwater depth was approximately 5 m deep. Fifteen sampling points were selected in each sampling area. In a given sampling period, three adult *P. euphratica* were selected at each sampling point, and 10 leaves were selected from each *P. euphratica* for spectral testing and field sample collection, which were conducted monthly from May 2016 to October 2016. In other words, 90 trees were selected from 30 sample points every month to collect 900 healthy leaves. The average spectral reflectance of 10 leaves of each *P. euphratica* was taken as the spectral reflectance value of one *P. euphratica*. At the same time, 10 leaves were mixed together to test the chlorophyll content, which was taken as the average chlorophyll content of one *P. euphratica*. Thus, there were spectral curves and chlorophyll content data for 90 trees per month. The leaves were immediately transported back to the laboratory in containers below 4°C for chlorophyll content analysis after spectral testing.

The spectral reflectance of *P. euphratica* leaves was measured by a portable field object spectral radiometer (ASD Fieldspec 4, Longmont, Colorado, USA) equipped with a barium sulfate standard whiteboard. Spectral reflectance data were obtained at an interval of 1.4 from 390 to 1100 nm by spectral testing. Reflectance was measured in 3 different parts of each leaf and then averaged as the spectral reflectance value of a leaf. The average spectral reflectance value of 10 leaves was taken as the final reflectance value of *P. euphratica* leaves at the sampling point.

Ten leaves of each tree collected were cut into fragments of 2×2 mm and mixed evenly. Approximately 100 mg of the fragments was put into a test tube with stopper containing the extracting solution and extracted at 4°C for 48 h. After the extraction was completed, the extracting solution was placed in a 1 cm quartz cuvette, and the absorbance of the solution at 537, 647, and 663 nm wavelength was determined by spectrophotometry (Shimadzu UV-2450, Kyoto, Japan). Using absorbance, the chlorophyll content was calculated by the following equations:

$$C_a\ (\mu\text{mol/mL}) = 0.01373A_{663} - 0.000897A_{537} - 0.003046A_{647}, \quad (1)$$

$$C_b\ (\mu\text{mol/mL}) = 0.024054A_{647} - 0.0004305A_{537} - 0.005507A_{663}, \quad (2)$$

$$\text{Chl}a\ (\text{mg/g}) = C_a M_a V/(1000 W_F), \quad (3)$$

$$\text{Chl}b\ (\text{mg/g}) = C_b M_b V/(1000 W_F), \quad (4)$$

$$\text{Chl}a + b\ (\text{mg/g}) = \text{Chl}a + \text{Chl}b, \quad (5)$$

where $C_a$ and $C_b$ are concentration of chlorophyll *a* and *b*, $A$ is absorbance, $V$ is volume of tissue extract (mL), $N$ is dilution coefficient, $M_a$ and $M_b$ are molecular weight of chlorophyll *a* and *b*, $W_F$ is fresh sample weight, Chl *a* and Chl *b* are chlorophyll *a* and *b* content calculated by fresh weight, and Chl *a*+*b* is the total chlorophyll content calculated by fresh weight.

First-order differential was used to process the spectral curve to highlight the differences in the spectral characteristics of the leaves in each month:

$$\rho'_{\lambda(i)} = (\rho_{\lambda(i+1)} - \rho_{\lambda(i-1)})/2\lambda\ , \quad (6)$$

where $\rho'_{\lambda(i)}$ is the first derivative value at wavelength $i$, $\rho_{\lambda(i+1)}$ and $\rho_{\lambda(i-1)}$ are the reflectance values at wavelengths $i$+1 and $i$–1, respectively, and $\lambda$ is wavelength intervals.

Continuum removal and smoothing of the spectral curve were performed to determine the position of the absorption trough. Spectral absorption characteristics are represented by wavelength at the lowest point of a trough ($\lambda_{BD}$), trough reflectance (ρ), trough depth ($H$), trough width ($W$), trough area ($A$), trough slope ($K$), trough symmetry ($S$), and spectral absorption characteristic index (SAI):

$$H = 1 - \rho_{Ci} \Big/ \left[ \rho_{Si} + \frac{\rho_{Ei} - \rho_{Si}}{\lambda_{Ei} - \lambda_{Si}} \left( \lambda_{Ci} - \lambda_{Si} \right) \right], \quad (7)$$

$$W = \lambda_{Ei} - \lambda_{Si}, \quad (8)$$

$$K = \tan^{-1} \left[ (\rho_{Ei} - \rho_{Si}) / (\lambda_{Ei} - \lambda_{Si}) \right], \quad (9)$$

$$A = \int_{\lambda_{Si}}^{\lambda_{Ei}} C\rho, \quad (10)$$

$$AL = \int_{\lambda_{Si}}^{\lambda_{BD}} C\rho, \quad (11)$$

$$S = AL/A, \quad (12)$$

$$SAI = \left[ W\rho_{SI} + (1 - W)\rho_{Ei} \right] / \rho, \quad (13)$$

where $\rho_{Ci}$, $\rho_{Si}$, and $\rho_{Ei}$ are the spectral reflectance of the center, starting point, and ending point of the trough, respectively; $\lambda_{Ci}$, $\lambda_{Si}$, and $\lambda_{Ei}$ are the wavelengths at the center, starting point, and ending point of the trough, respectively; $A$ and $AL$ are the area of the trough and the area to the left of the trough, respectively; $C\rho$ is the value after continuum removal was performed; $\lambda_{BD}$ is the wavelength at the lowest point of a trough; ρ is the reflectance at the lowest point of a trough.

The model was fitted by partial least squares regression (PLSR), and the accuracy verification indicators were the root mean square error (RMSE), mean relative error (MRE), and standard deviation to the root mean square error ratio (RPD):

$$\text{RMSE} = \sqrt{\sum_{i=1}^{n} (y_i - y_i')^2 / n}, \quad (14)$$

$$\text{MRE} = \frac{y_i - y_i'}{y_i'} \frac{1}{n}, \quad (15)$$

$$\text{RPD} = \text{SD}'/\text{RMSE}, \quad (16)$$

where $y_i$ is the measured value, $y_i'$ is the predicted value, $n$ is the number of samples, and SD′ is the standard deviation of the predicted values.

**Results and discussion.** The chlorophyll contents of healthy and water-stressed *P. euphratica* leaves from May to October were determined. Characteristics of chlorophyll content changes in leaves of *P. euphratica* were analyzed.

Statistics analyses showed that the range of chlorophyll content in leaves of healthy and water stressed *P. euphratica* from May to October. The range of Chl *a*, Chl *b*, Chl *a*+*b*, and Chl *a*/Chl *b* in leaves of healthy *P. euphratica* were 0.27±0.09–1.25±0.16 mg/g, 0.09±0.04–0.41±0.06 mg/g, 0.36±0.12–1.65±0.24 mg/g, and 3.14±0.43–5.17±0.53 mg/g, respectively, but the range of Chl *a*, Chl *b*, Chl *a*+*b*, and Chl *a*/Chl *b* in leaves of water-stressed *P. euphratica* were 0.10±0.04–0.89±0.12 mg/g, 0.03±0.01–0.27±0.04 mg/g, 0.13±0.05–1.15±0.15 mg/g, and 3.22±0.13–5.11±0.41 mg/g, respectively. Difference significance test indicated that the chlorophyll content of leaves of healthy *P. euphratica* was significantly higher than that of water stress ($P \leq 0.01$),while the ratio of Chl *a* to Chl *b* of healthy *P. euphratica* leaves from June to August was significantly different from that of water stressed *P. euphratica* leaves ($P \leq 0.05$), but there was no significant difference for Chl *a*/Chl *b* in May, September and October ($P > 0.05$).

The efficiency of photosynthesis depends mainly on pigments. The chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*) and Chl *a*/Chl b ratio have a direct influence on the photosynthetic rate [12]. Figure 1 indicates that the Chl *a*, Chl *b*, and Chl *a*+*b* in the leaves of healthy and water-stressed *P. euphratica* from May to October showed a trend of increasing first and then decreasing. Among them, the chlorophyll contents in July and August were higher than those in other months, and the lowest was in October, which showed that the photosynthetic efficiency also showed a trend of increasing first and then decreasing. Overall, the chlorophyll content of healthy *P. euphratica* leaves was higher than that of water-stressed *P. euphratica* leaves, which indicated that the photosynthesis efficiency of healthy *P. euphratica* in the whole growing season was better than that in the water-stress leaves. The difference in the Chl *a*/Chl *b* from May to August was not significant for healthy and water-stressed *P. euphratica* leaves. The Chl *a*/Chl *b* in September and October was significantly higher than that in the previous four months ($P < 0.01$), with the highest Chl a/Chl b in October. Although the chlorophyll content and Chl *a*/Chl *b* of healthy *P. euphratica* leaves were higher than those of water-stressed *P. euphratica* leaves, the regularity of their changes with seasonal changes was consistent. Changes in the chlorophyll content and Chl *a*/Chl *b* can reflect that the photosynthetic function of plant leaves is strong or weak and can also represent the aging status of plant tissues or organs [13]. Therefore, the study used September as the inflection point of the photosynthetic efficiency attenuation of *P. euphratica*. Because *P. euphratica* is a C3 plant, the Chl *a*/Chl *b* should generally be approximately 3 but increased to 5.10 in October, indicating that the function of *P. euphratica* leaves was abnormal in October.

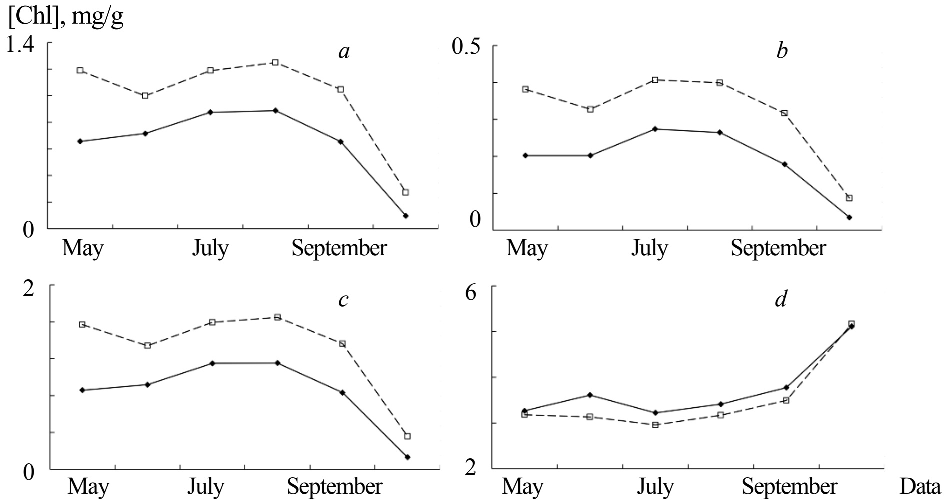


Fig. 1. Changes in (a) Chl *a*, (b) Chl *b*, (c) Chl *a*+*b*, and (d) Chl *a*/Chl *b* in leaves of healthy *Populus euphratica* (□) and water-stressed *Populus euphratica* (◆) from May to October, demonstrating that Chl *a*, Chl *b*, and Chl *a*+*b* show a trend of increasing first and then decreasing, but Chl *a*/Chl *b* show a continuous increasing trend.

The absorption characteristics of the spectral curves of *P. euphratica* leaves were analyzed after continuum removal and smoothing. The absorption, reflection, and emission characteristics of spectral curves are highlighted effectively by continuum removal and unify the spectral curves to a consistent spectral background. It is helpful to compare the characteristic values with those of other spectral curves. The noise of the spectral curves was eliminated by smoothing (Fig. 2). Figure 2 shows that there are two main absorption troughs: one is at ~500 nm, and the other is at ~675 nm.

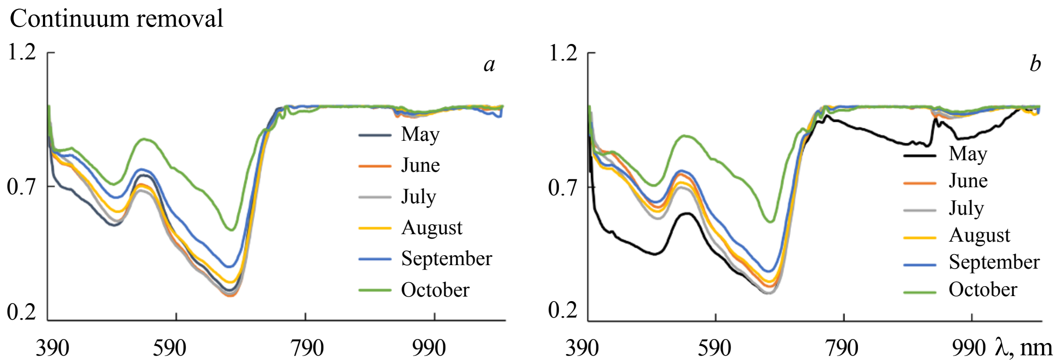


Fig. 2. Spectral curves of (a) healthy and (b) water-stressed *Populus euphratica* leaves between 395 and 1100 nm after continuum removal and smoothing from May to October, demonstrating that these two types of curves have two main absorption troughs near the wavelengths of 500 and 675 nm.

*Spectral absorption characteristics of healthy and water-stressed P. euphratica leaves near the wavelength of 500 nm.* After envelope removal and smoothing, there was an absorption valley in the spectrum curve of *Populus euphratica* leaves near the wavelength of 500 nm, and nine parameters related to this absorption valley were analyzed. After continuum removal and smoothing, the parameter variation characteristics of the absorption trough near 500 nm from May to October were analyzed, which indicated that $\lambda_{BD}$, *H*, *S*, and SAI appear to increase first and then decrease, while ρ, *W*, and *K* show a trend of decreasing first and then increasing, and *A* and *AL* show a continuous decreasing trend.

Table 1 shows that for the absorption valley near the wavelength of 500 nm, $\lambda_{BD}$, *H*, *S*, and SAI appeared to increase first and then decrease, while ρ, *W*, and *K* showed a trend of decreasing first and then increasing. Because Chl a, Chl b, and Chl a+b showed a trend of increasing first and then decreasing from May to October, the sizes of these seven absorption characteristic parameters have a certain correlation with the chlorophyll content in the leaves. However, *AL* and *A* show no obvious change characteristics. The difference in $\lambda_{BD}$ was not significant ($P > 0.05$); the differences in ρ, *H*, *K*, and SAI were very significant ($P < 0.01$); the differences in *W*, *AL*, *A*, and *S* were significant in May ($P < 0.05$); the differences in $\lambda_{BD}$, *W*, *A*, and *S* were not significant ($P > 0.05$); the differences in ρ, *H*, and SAI were very significant ($P < 0.01$); the differences in *K* and *AL* were significant ($P < 0.05$) in June; the differences in all parameters from July to October were not significant ($P > 0.05$) between healthy and water-stressed *Populus euphratica* leaves.

TABLE 1. Absorption Characteristics of Healthy and Water-Stressed *Populus euphratica* Leaves near 500 nm Wavelength during Different Months

| Plant status | Month | $\lambda_{BD}$ | ρ | *W* | *H* | *AL* | *A* | *K* | *S* | SAI |
|---|---|---|---|---|---|---|---|---|---|---|
| Healthy | May | 494 | 0.55 | 144.40 | 0.45 | 22.88 | 29.45 | –0.0018 | 0.78 | 78.24 |
| | June | 498 | 0.56 | 142.19 | 0.44 | 19.18 | 23.43 | –0.0021 | 0.81 | 76.80 |
| | July | 499 | 0.56 | 141.88 | 0.44 | 18.08 | 21.91 | –0.0023 | 0.81 | 85.42 |
| | August | 499 | 0.60 | 142.36 | 0.40 | 17.34 | 20.73 | –0.0021 | 0.83 | 74.93 |
| | September | 497 | 0.65 | 142.36 | 0.35 | 14.99 | 18.61 | –0.0017 | 0.79 | 55.24 |
| | October | 493 | 0.69 | 146.34 | 0.32 | 15.48 | 20.76 | –0.00094 | 0.74 | 30.88 |
| Water-stressed | May | 494 | 0.44 | 149.93 | 0.56 | 33.55 | 40.59 | –0.0028 | 0.82 | 155.51 |
| | June | 499 | 0.62 | 141.26 | 0.38 | 15.29 | 18.77 | –0.0018 | 0.81 | 60.46 |
| | July | 499 | 0.57 | 141.93 | 0.43 | 16.76 | 20.63 | –0.0022 | 0.81 | 79.82 |
| | August | 499 | 0.60 | 143.34 | 0.40 | 18.23 | 21.91 | –0.0020 | 0.82 | 72.19 |
| | September | 495 | 0.63 | 143.25 | 0.37 | 17.08 | 21.29 | –0.0017 | 0.79 | 59.23 |
| | October | 492 | 0.69 | 147.93 | 0.31 | 17.41 | 23.15 | –0.0007 | 0.75 | 24.82 |

N o t e. After continuum removal and smoothing, the parameter variation characteristics of the absorption trough near 675 nm from May to October were analyzed, which indicated that *W*, *H*, *AL*, *A*, and *K* appear to increase first and then decrease, *P* and SAI tend to decrease first and then increase, while $\lambda_{BD}$ and *S* show no obvious change in trend.

Table 2 shows that the $\lambda_{BD}$, *H*, *S*, and SAI had a significant or extremely significant positive correlation ($P < 0.05$ or $P < 0.01$) with chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*), while ρ, *W*, *K*, and chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*) showed a significant negative correlation ($P < 0.01$) for the absorption valley near the wavelength of 500 nm. Among them, the correlation coefficient between K and chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*) was larger than the others, reaching 0.5 and 0.6. There was no significant correlation ($P > 0.05$) between *AL* and *A* and chlorophyll content (Chl a, Chl b, Chl a+b), and there was no significant correlation 500-7 5) between all absorption parameters and Chl *a*/Chl *b*. After continuum removal and smoothing, the spectrum curve of *Populus euphratica* leaves near the wavelength of 675 nm also had an absorption valley, and nine parameters related to this absorption valley were analyzed.

Table 3 shows that *W*, *H*, *AL*, *A*, and *K* appear to increase first and then decrease, and *P* and SAI tend to decrease first and then increase, which should be closely related to the chlorophyll content of *P. euphratica* leaves. From May to October, the chlorophyll content showed a trend of increasing first and then decreasing, while $\lambda_{BD}$ and *S* showed no obvious change trend. The differences in ρ, $\lambda_{BD}$ and *W* were not significant ($P > 0.05$); the differences in *H*, *K*, *AL*, and *A* were very significant ($P < 0.01$); the differences in A and SAI were significant in May ($P < 0.05$); the differences in *W* and *S* were not significant ($P > 0.05$); the differences

TABLE 2. Correlation between Spectral Absorption Parameters of Absorption Trough near 500 nm Wavelength and Chl *a*, Chl *b*, Chl *a*+*b*, and Chl *a*/Chl *b*

| Plant status | Chlorophyll type | $\lambda_{BD}$ | ρ | *W* | *H* | *AL* | *A* | *K* | *S* | SAI |
|---|---|---|---|---|---|---|---|---|---|---|
| Healthy | Chl *a* | 0.316* | –0.409** | –0.347** | 0.323* | 0.111 | 0.031 | –0.540** | 0.385** | 0.359** |
| | Chl *b* | 0.309* | –0.418** | –0.335** | 0.333** | 0.112 | 0.036 | –0.526** | 0.372** | 0.347** |
| | Chl *a*+*b* | 0.318* | –0.419** | –0.347** | 0.332** | 0.116 | 0.035 | –0.545** | 0.389** | 0.362** |
| | Chl *a*/Chl *b* | 0.098 | 0.054 | –0.054 | 0.152 | –0.058 | –0.074 | –0.097 | 0.184 | 0.015 |
| Water–Stressed | Chl *a* | 0.481** | –0.363** | –0.313* | 0.361** | –0.025 | –0.076 | –0.604** | 0.398** | 0.361** |
| | Chl *b* | 0.495** | –0.393** | –0.284* | 0.391** | 0.011 | –0.044 | –0.633** | 0.431** | 0.390** |
| | Chl *a*+*b* | 0.487** | –0.372** | –0.308* | 0.370** | –0.016 | –0.069 | –0.614** | 0.408** | 0.370** |
| | Chl *a*/Chl *b* | –0.126 | 0.245 | 0.212 | –0.244 | –0.079 | –0.031 | 0.467** | –0.322 | –0.325* |

* Indicates that the concomitant probability value is less than or equal to 0.05, and the correlation is significant.
** Indicates that the concomitant probability value is less than or equal to 0.01, and the correlation is very significant.

TABLE 3. Absorption Characteristic of *Populus euphratica* Leaves near 675 nm Wavelength during Different Months

| Plant status | Month | $\lambda_{BD}$ | ρ | *W* | *H* | *AL* | *A* | *K* | *S* | SAI |
|---|---|---|---|---|---|---|---|---|---|---|
| Healthy | May | 672 | 0.31 | 223.44 | 0.65 | 42.89 | 64.51 | 0.0011 | 0.66 | –182.87 |
| | June | 673 | 0.29 | 228.40 | 0.67 | 44.43 | 67.53 | 0.0013 | 0.66 | –231.61 |
| | July | 673 | 0.30 | 229.02 | 0.66 | 42.89 | 66.22 | 0.0014 | 0.65 | –250.26 |
| | August | 674 | 0.34 | 228.82 | 0.61 | 39.84 | 61.00 | 0.0013 | 0.65 | –203.23 |
| | September | 672 | 0.40 | 226.95 | 0.56 | 35.81 | 55.32 | 0.0011 | 0.65 | –136.05 |
| | October | 675 | 0.53 | 221.34 | 0.44 | 26.98 | 41.81 | 0.0006 | 0.65 | –58.00 |
| Water-stressed | May | 672 | 0.29 | 219.77 | 0.63 | 33.86 | 52.51 | 0.0015 | 0.64 | –252.83 |
| | June | 674 | 0.33 | 228.03 | 0.63 | 43.06 | 65.30 | 0.0011 | 0.66 | –176.94 |
| | July | 673 | 0.30 | 228.88 | 0.65 | 42.68 | 65.26 | 0.0014 | 0.65 | –232.65 |
| | August | 674 | 0.34 | 228.23 | 0.61 | 39.92 | 61.06 | 0.0013 | 0.66 | –193.55 |
| | Sept. | 672 | 0.38 | 223.51 | 0.57 | 36.73 | 57.03 | 0.0011 | 0.64 | –144.90 |
| | Oct. | 675 | 0.57 | 220.72 | 0.41 | 23.06 | 35.58 | 0.0005 | 0.65 | –42.53 |

N o t e. After continuum removal and smoothing, the parameter variation characteristics of the absorption trough near 675 nm from May to October were analyzed, which indicated that *W*, *H*, *AL*, *A*, and *K* appear to increase first and then decrease, *P* and SAI tend to decrease first and then increase, while $\lambda_{BD}$ and *S* show no obvious change in trend.

in ρ, *H*, *AL*, *A*, and SAI were very significant ($P < 0.01$); the differences in $\lambda_{BD}$ and *K* were significant ($P < 0.05$) in June; the differences in all parameters from July to October were not significant ($P > 0.05$) between healthy and water-stressed *Populus euphratica* leaves.

Table 4 shows that H, AL, A, and K had a significant or extremely significant positive correlation ($P < 0.05$ or $P < 0.01$) with chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*), while $\lambda_{BD}$, ρ, and SAI chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*) showed a significant negative correlation ($P < 0.01$) for the absorption valley near the wavelength of 675 nm. Among them, the correlation coefficient between *H* and chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*) was larger than the others, reaching 0.76 and 0.86, respectively. There was no significant correlation ($P > 0.05$) between *W* of healthy *P. euphratica* leaves, *S* of water-stressed *P. euphratica* leaves and chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*). The Chl *a*/Chl *b* of leaves of healthy *P. euphratica* was positively correlated with K and negatively correlated with S. The Chl *a*/Chl *b* of leaves of water-stressed *P. euphratica* was negatively correlated with *H*, *A*, *AL*, *K*, and *W* and positively correlated with ρ and SAI. The correlation between the chlorophyll content of healthy *P. euphratica* leaves and the absorption parameters of the absorption valley near the 675 nm wavelength was generally lower than that of water-stressed *P. euphratica* leaves.

Estimation models for the chlorophyll content of *Populus euphratica* leaves were established by partial least squares regression (PLSR). PLSR is one of the modeling methods to solve high throughput data, which introduces a considerable number of variable selection methods. PLSR integrates multiple linear regression, principal component analysis, and canonical correlation analysis; it can partially eliminate the multicollinearity between variables and reduce the deviation of factor contributions to the results; at the same time, under the condition that the number of sample points is less than the number of variables, regression modeling can also be performed [14]. Thus, PLSR is widely used in various data modeling, such as crop yield estimation, spectral modeling, remote sensing image processing, etc. [15–17]. In this study, the samples were randomly divided into two groups. Two-thirds of the samples was used for modeling, and the remaining one-third was used to validate the model. There was an estimation model between the absorption characteristic parameters and chlorophyll content using PLSR. The latent variables were computed for each model using iterative methods as linear combinations of the independent variables (absorption characteristic parameters) and dependent variables (each chlorophyll content measured in the laboratory). Therefore, each PLSR defined a set of latent variables, maximized the covariance between the two original spaces, and sought to achieve an optimal prediction for new data. The new variables were identified, representing latent variables or estimates of their rotation [3].

TABLE 4. Correlation between Spectral Absorption Parameters of Absorption Trough near 675 nm Wavelength and Chl *a*, Chl *b*, Chl *a*+*b*, and Chl *a*/Chl *b*

| Plant status | Chlorophyll type | $\lambda_{BD}$ | $\rho$ | $W$ | $H$ | $AL$ | $A$ | $K$ | $S$ | SAI |
|---|---|---|---|---|---|---|---|---|---|---|
| Healthy | Chl *a* | $-0.392^{**}$ | $-0.759^{**}$ | 0.171 | $0.765^{**}$ | $0.709^{**}$ | $0.658^{**}$ | $0.518^{**}$ | $0.303^{*}$ | $-0.563^{**}$ |
| | Chl *b* | $-0.338^{**}$ | $-0.735^{**}$ | 0.218 | $0.748^{**}$ | $0.700^{**}$ | $0.647^{**}$ | $0.514^{**}$ | $0.314^{*}$ | $-0.582^{**}$ |
| | Chl *a*+*b* | $-0.382^{**}$ | $-0.761^{**}$ | 0.185 | $0.769^{**}$ | $0.715^{**}$ | $0.662^{**}$ | $0.523^{**}$ | $0.309^{*}$ | $-0.574^{**}$ |
| | Chl *a*/Chl *b* | 0.077 | 0.032 | 0.050 | –0.090 | –0.011 | 0.183 | $0.255^{*}$ | $-0.678^{**}$ | –0.141 |
| Water-stressed | Chl *a* | $-0.426^{**}$ | $-0.830^{**}$ | $0.498^{**}$ | $0.866^{**}$ | $0.842^{**}$ | $0.848^{**}$ | $0.680^{**}$ | –0.076 | $-0.697^{**}$ |
| | Chl *b* | $-0.392^{**}$ | $-0.814^{**}$ | $0.504^{**}$ | $0.841^{**}$ | $0.792^{**}$ | $0.795^{**}$ | $0.698^{**}$ | –0.059 | $-0.722^{**}$ |
| | Chl *a*+*b* | $-0.420^{**}$ | $-0.830^{**}$ | $0.502^{**}$ | $0.865^{**}$ | $0.835^{**}$ | $0.840^{**}$ | $0.688^{**}$ | –0.073 | $-0.707^{**}$ |
| | Chl *a*/Chl *b* | 0.250 | $0.450^{**}$ | $-0.271^{*}$ | $-0.450^{**}$ | $-0.424^{**}$ | $-0.412^{**}$ | $-0.397^{**}$ | –0.042 | $0.376^{**}$ |

$^{*}$Indicates that the concomitant probability value is less than or equal to 0.05, and the correlation is significant.
$^{**}$Indicates that the concomitant probability value is less than or equal to 0.01, and the correlation is very significant.

Because some absorption characteristics of the absorption trough have a certain correlation with the chlorophyll content, this study attempts to establish some chlorophyll content estimation models using these parameters. Table 5 shows that at the absorption trough near the wavelength of 500 nm, only the Chl *b* estimation model of the water-stressed *P. euphratica* leaves is robust, and the estimation models of the other chlorophyll types do not meet the accuracy requirements. Here $R^2$ and RPD can reflect the correlation between the predicted value and the measured value, and RMSE and MRE can reflect the deviation between the predicted value and the measured value. The larger the values of $R^2$ and RPD, the smaller the values of RMSE and MRE, indicating that the performance of the model is better and the prediction ability is stronger. For RPD, when RPD $\geq$ 2, it indicates that the model has good predictive ability. When $1.4 \leq$ RPD $< 2.0$, the model has a rough estimation ability. When RPD $< 1.4$, it indicates that the model does not have the ability to predict [18]. In Table 5, the fitting $R^2$ and predicted $R^2$ of the Chl *b* estimation model of the water-stressed *P. euphratica* leaves are higher, the RPD is greater than twice the values of the other models, and the MRE is less than 10%. The remaining models have either an MRE greater than 10% or an RPD less than 1.4, which do not meet the accuracy requirements. By modeling the chlorophyll content and the absorption characteristic parameters of the absorption trough near the wavelength of 675 nm, it was found that the estimation models of Chl *a*, Chl *b*, and Chl *a*+*b* of water-stressed *P. euphratica* leaves met the requirements of accuracy and could roughly estimate the chlorophyll content of water-stressed *P. euphratica* leaves.

Photosynthesis is a very important physiological process in plant growth and development [19]. Chlorophyll is a very important photosynthetic pigment. With the change in seasons, plant chlorophyll content-influencing factors such as temperature, humidity, soil moisture, light and plant physiological, and structural factors are constantly changing, and these changes will inevitably cause changes in plant photosynthetic characteristics. Chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b*) in the leaves of healthy and water-stressed *P. euphratica*

TABLE 5. Model Parameters of PLSR between Chlorophyll Content and Absorption Characteristics of Absorption Trough near 500 and 675 nm Wavelength

| Plant status | Chlorophyll type | Fitting $R^2$ | Predicted $R^2$ | RMSE | RMSEP | MRE | RPD |
|---|---|---|---|---|---|---|---|
| λ = 500 nm | | | | | | | |
| Healthy | Chl *a* | 0.693 | 0.560 | 0.191 | 0.223 | 3.31 | 1.31 |
| | Chl *b* | 0.646 | 0.482 | 0.072 | 0.482 | 6.85 | 1.18 |
| | Chl *a*+*b* | 0.694 | 0.555 | 0.256 | 0.300 | 3.90 | 1.31 |
| | Chl *a*/Chl *b* | 0.769 | 0.596 | 1.308 | 1.729 | 5.93 | 1.45 |
| Water-stressed | Chl *a* | 0.691 | 0.490 | 0.155 | 0.198 | 186.51 | 1.63 |
| | Chl *b* | 0.714 | 0.558 | 0.046 | 0.057 | 3.58 | 5.07 |
| | Chl *a*+*b* | 0.703 | 0.515 | 0.197 | 0.252 | 29.05 | 1.47 |
| | Chl *a*/Chl *b* | 0.107 | 0.007 | 1.963 | 2.070 | / | / |
| λ = 675 nm | | | | | | | |
| Healthy | Chl *a* | 0.706 | 0.623 | 0.193 | 0.218 | 0.500 | 1.35 |
| | Chl *b* | 0.700 | 0.614 | 0.067 | 0.075 | 0.358 | 1.33 |
| | Chl *a*+*b* | 0.719 | 0.636 | 0.250 | 0.284 | 0.512 | 1.39 |
| | Chl *a*/Chl *b* | 0.021 | –0.082 | 2.371 | 2.491 | – | – |
| Water-stressed | Chl *a* | 0.766 | 0.743 | 0.135 | 0.141 | 4.254 | 1.75 |
| | Chl *b* | 0.728 | 0.703 | 0.044 | 0.046 | 3.238 | 1.6 |
| | Chl *a*+*b* | 0.764 | 0.741 | 0.176 | 0.184 | 4.122 | 1.74 |
| | Chl *a*/Chl *b* | 0.144 | 0.021 | 1.874 | 2.004 | – | – |

N o t e. The closer $R^2$ is to 1, the better the fitting effect of the model. When RMSE and MRE are smaller, the regression model is better. RPD is greater than 1.8, indicating that the model is acceptable. According to these model parameters, one can judge whether the accuracy of the model is acceptable.

showed a trend of first decreasing, then increasing and then decreasing with seasonal change. From May to October, the chlorophyll content of healthy *P. euphratica* leaves was higher than that of water-stressed *P. euphratica*, while the ratio of Chl *a*/Chl *b* in healthy *P. euphratica* leaves was lower than that of water-stressed *P. euphratica* from June to September. Studies have shown that water stress can reduce the content of photosynthetic pigments in plants and increase the content of proline, malondialdehyde, lipoxygenase, phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase, and the decrease of Chl *b* is more than the decrease of Chl *a*. Therefore, the Chl *a*/Chl *b* value of the leaves of plants under water stress is greater than that of healthy plants [20–22]. Naghizadeh et al. [23] showed that water stress decreased Chl *a*, Chl *b* and Car by approximately 48.4, 73.15, and 90.2%, respectively, compared to nontreated plants. Thus, studies on chlorophyllase and peroxidase have shown that the reduction of chlorophyll is more likely to accelerate the degradation of chlorophyll rather than slow down its synthesis [24]. Wang et al. [25] found that the chlorophyll content of *P. euphratica* leaves decreased with decreasing groundwater levels, while the ratio Chl *a*/Chl *b* increased with decreasing groundwater. The Chl *a*, Chl *b*, Chl *a*+*b* and Chl *a*/Chl *b* of *P. euphratica* leaves in areas with high groundwater levels in August were 1.37, 0.44, 1.81, and 3.11 mg/g, respectively, while the Chl *a*, Chl *b*, Chl *a*+*b*, and Chl *a*/Chl *b* of *P. euphratica* leaves in areas with low groundwater levels were 0.95, 0.24, 1.19, and 3.92 mg/g, respectively, in Awat County of Xinjiang, China. Yuan et al. [26] showed that in the Aibi Lake area of Xinjiang, China, Chl *a* and Chl *b* decreased gradually, Chl *b* decreased greatly, and Chl *a*/Chl *b* increased gradually with increasing drought stress. Meanwhile, when drought stress was found, Chl *a* and Chl *b* were 1.2 and 0.4, respectively. When there is no drought stress, Chl *a* and Chl *b* were 0.8 and 0.2, respectively. These results are close to the results of this study. To date, there is no research on the seasonal variation in chlorophyll in the leaves of healthy *P. euphratica* and water-stressed *P. euphratica*. At present, studies on chlorophyll change characteristics in leaves of *P. euphratica* have mainly focused on chlorophyll change characteristics under stress [27, 28]. Revealing the seasonal variation in the chlorophyll content in leaves of *P. euphratica* can also provide a theoretical basis for the ecological conservation of *P. euphratica* in arid areas. Hu et al. [29] studied the change characteristics of chlorophyll in the leaves of jujube trees and concluded that the chlorophyll showed a continuous increasing trend from May to October. Because of different plant species, the results of this study were inconsistent with the results of this study. In recent years, studies on the spectral characteristics of *P. euphratica* leaves have focused on the spectral inversion of physiological parameters, the spectral curve characteristics of leaves under stress, and the spectral analysis of desert vegetation [30, 31]. However, there are few studies on the spectral characteristics of *P. euphratica* leaves in different seasons. Nyongesah et al. [32] estimated the Chl *a*/Chl *b* ratio through PRI, which can provide important information for the study of the carbon balance and stress response of desert plants. The present study did not involve the seasonal variation characteristics of chlorophyll and spectrum in healthy *P. euphratica* leaves and water-stressed *P. euphratica* leaves. These two aspects are what we must grasp in the study of the evolution of desert riparian ecosystems by remote sensing. The seasonal variation in chlorophyll is the basis for the formulation of *P. euphratica* protection measures and the key factor for *P. euphratica* health evaluation, which is also of significance in this study.

**Conclusions.** The seasonal variation characteristics of the chlorophyll content and spectral reflectance of healthy *P. euphratica* and water-stressed *P. euphratica* leaves were analyzed. The results showed that from May to October, the chlorophyll *a* content, chlorophyll *b* content, and total chlorophyll content all presented in the form of "two peaks and two valleys," and the chlorophyll content of healthy *P. euphratica* leaves was higher than that of water-stressed *P. euphratica* leaves. However, the Chl *a*/Chl *b* of water-stressed *P. euphratica* leaves was higher than that of healthy *P. euphratica* leaves in the growing season. In some wavelength range, the spectral reflectance difference in different months was significantly. The spectral curves of healthy *P. euphratica* leaves from May to August were relatively consistent, and the curves in the near-infrared band were more concentrated than that of the water-stressed *P. euphratica* leaves. Two absorption troughs were observed near the wavelengths of 500 and 675 nm. The absorption characteristic parameters of the absorption trough had a certain correlation with the chlorophyll content. The PLSR model established by using the absorption parameters near the wavelengths of 500 nm can roughly estimate Chl *b* of water-stressed *P. euphratica* leaves, and the PLSR model constructed by the absorption parameters near the wavelengths of 675 nm can roughly estimate the chlorophyll content (Chl *a*, Chl *b*, and Chl *a*+*b*) of water-stressed *P. euphratica* leaves.

**Acknowledgments.** This study was supported financially by the National Natural Science Foundation of China (31860172, 41877071, 31260140) and Xinjiang Production and Construction Corps Key Field Innovation Team Building Plan (2018CB003).

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