Non-destructive Analysis Chlorophyll Content of Different Genotypes of Poplars Based on Hyperspectral Reflectance Data

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Abstract. Leaf Chlorophyll content (Ct) indicates plant physiological status and can be detected by hyperspectral measurements. However, it is difficult to conclude that different genotypes of same species have the same relationship with the hyperspectral data. The aim of this paper was to test that whether the different genotypes of same species have the similar relationship with hyperspectral reflectance. First of all, spectral reflectance of populus simonii (Populus simonii Carr) and I-72 poplar (Populus euramericana cv. ‘San Martino I-72/58’) were collected by spectrometric meter, and then extract chlorophyll index (CI) and other 11 types of vegetation indices from the hyperspectral reflectance data. At last, relationships between different vegetation indices and Ct of the two genotypes of poplar were compared. Results show that (1) the relationships between SPAD value and Ct are different in the low and high Ct level, we can choose proper vegetation index, REPIG, mSR705 and SDr/SDb et al to predict the Ct value. (2) Meanwhile, we can use PSSRb and PRI to distinguish fine difference between different genotypes.

1. Introduction
In the process of light energy absorption of photosynthesis, chlorophyll absorbs light energy and has a direct relationship with the energy use of vegetation. Chlorophyll content has a good correlation with photosynthetic capacity of vegetation, developmental stages and nitrogen status, they can usually indicate nitrogen stress, photosynthetic capacity and vegetation growth phase (especially the aging stage) [1]. Using spectrophotometer in lab to determinate chlorophyll content is a traditional method. It’s a time consuming method and also damage causing to vegetation. In addition, it is likely to loss chlorophyll in the process of transportation from the field to the laboratory and sample preparation [2]. Thus, quick and accurate measurement of plant leaf chlorophyll content has important application value in many areas such as ecology, agriculture and forestry [3].

For the purpose of fast and non-destructive determination of content of vegetation pigment and other biochemical components, there are some devices such as SPAD 502 and method for rapid determination of chlorophyll relative content value of vegetation leaf [4]. In the recent years, hyperspectral technology develops rapidly. Due to its more and narrower band, it provides a more convenient, fast, non-destructive method for collecting data and estimating leaf or canopy absolute chlorophyll content [5]. Through the determination of the green leaf reflectance, transmittance and
absorption, Portable hyper-spectrometer is a non-invasive method of determination of chlorophyll, which makes the hyperspectral technology has a unique role in vegetation chlorophyll content evaluation. Portable spectrometer uses its delicate high spectral resolution, establishing regression equation between chlorophyll content and spectral reflectance in some characteristic bands, as the basis of prediction. Vegetation spectral index is based on the assumption that vegetation spectral behavior, unique biophysical function and biological physical properties are related, it is likely to estimate vegetation parameter more accurately, and reduce the influence of external factors. Therefore, vegetation spectral information characteristics can be used to estimate chlorophyll content [6-7]. Gitelson et al. find there is a significant correlation between chlorophyll content and C\textsubscript{rededge} based on three bands of vegetation index, $R^2$ is more than 0.9[8]. Horler et al. show that reflection spectrum in the red edge position may indicate the change of vegetation chlorophyll concentration [9]. Because the first order differential data of the red edge is discrete, the red edge position extracted from traditional algorithm causes larger deviation on chlorophyll content. Miller et al. [10] and Cho and Skidmore [11] respectively use Gaussian optical model and linear extrapolated method to successfully correct a red edge location algorithm, which has achieved good effect on the estimation of chlorophyll. Blackburn [12-13] find that Canopy and leaf scales pigment concentration have extremely significant correlation with the first and second order derivative of the spectral reflectance, and construct the suitable chlorophyll spectral index for estimating chlorophyll by using the characteristic absorption bands of Chlorophyll in 470nm, 635nm and 680nm. However, Sims and Gamon [14] suggest that the reflectance of 550nm, 700nm and 680nm are more sensitive to chlorophyll from his further study. There are other vegetation indices such as NDVI, RVI and PRI are often used for remote sensing of chlorophyll density monitoring, and all have good results [15]. Current research on chlorophyll high spectral estimation has made great progress, the spectral parameters method considers its physical mechanism in constructing index, at the same time, requires less band, leading to its widespread use[16]. But even so, the spectral index is influenced by vegetation type, developmental stage, background environment and other factors[17]. Its sensitivity to the different vegetation, different growth period and internal theory remain to be further in-depth study.

There are many studies focus on the single crop, such as rice, corn, and soybean and so on, but lack of the analysis of different forest tree species, especially plants of different genotypes. We need to know whether they have similar patterns between two different genotypes, although they are both poplars. On the foundation of these, this paper chooses two different genotypes of poplars and tries to study whether hyperspectral reflectance data can apply to non-destructive test of chlorophyll content, and answer the pattern is similar or not.

2. Materials and Methods

2.1. The study area and Materials collection

Research was conducted at Huazhong agricultural university campus, Wuchang district of Wuhan city, Hubei province (30° 28’ 57.79 ″ N, 114° 21’ 37.23” E). Populus simonii (Populus simonii Carr) and I-72 poplar (Populus euramericana cv. ‘San Martino I-72/58’) are selected as the experimental materials. Collecting different ages leaves in Windless cloudy weather or sunny in the morning or the evening. We choose 3 trees of each poplar, and use long reach chain saw to get 4 branches of every tree in 4 different directions in the intermedial height. Selecting leaves from the top branch to the bottom, then measure the SPAD value of every leaves. Ensuring leaves include different age stages and are all normal growth, free from disease, insect. Then choose proper leaves and put them into the ice box with the value bag.

2.2. Spectrum data collection

Spectrum data was collected through PSR-3500 Spectral Evolution. The spectrometer wavelength range is 350 ~ 2500nm and spectral resolution is 3.5nm (350-1000nm), 10nm @ 1500nm, 7nm @ 2100nm. Before using leaf clip to determinate spectrum, instrument need to be optimized by using the
white plate side. While measuring, put the leaf between the leaf clips, whose underlying surface is black. Keeping the leaf fill the whole viewing angle, avoid leaf veins, test 6 times per leaf and 10 spectrum curves per times. The average 10 sets of data as the spectral reflectance of each time, the average data of 6 times as the sample final spectral reflectance. The instrument needs to be optimized at intervals of about 10 minutes.

2.3. Chlorophyll content data collection
The absolute chlorophyll content of per leaf was measured by traditional alcohol extraction method. Punching every measured leaf to get as many disks (n) with a puncher whose diameter is 0.87 cm, calculating those disks total area and grind them with 95% alcohol, SiO2, CaCO3, filtering and keeping constant volume to 25 ml, measuring absorbance of chlorophyll a and b at the wavelength of 665 nm and 649 nm respectively by using spectrophotometer of UNICO-UV 2102C, finally converting to their absolute content (mg/cm^2) according to the equation 1.

\[ Ca = 13.95A_{665} - 6.88A_{649} \]
\[ Cb = 24.96A_{649} - 7.32A_{665} \]
\[ Ct = (Ca + Cb) \times \frac{25 \times (10)^{-3}}{n \times \Pi \times (0.87)^2} \]

Where n is the number of disk, Ct is the total content of chlorophyll (mg/cm^2).

2.4. Vegetation index and Model verification
Numerous vegetation indices have been used to study the relation between spectral index and chlorophyll. This research chooses the following spectral indices, Chlorophyll index (CI) [8], Pigment specific simple ratio (PSSRb) [12], Simple ratio index modified on SR705 (mSR705) [14], Normalized difference index(ND705)[15], Simple ratio index(SR)[15], Photosynthetic reflectance index(PRI)[15], Ratio vegetation index(RVI)[18], Ratio of reflectance at 750 nm to 550 nm(GMI) [19], Red edge reflectance ratio index(VOG3) [20], Ratio of reflectance at 750 nm to 710 nm(ZM)[21], Radio of the red-edge integral areas and the blue-edge integral areas(SDr/SDb) [22], Red edge position obtained from inverted gaussian fitting technique(REPIG) [23].

Randomly select 2/3 the total sample to build regression equations, and the other as the verification sample. We use the verification sample to calculate y’ according to the regression equation, which is the estimated value of actual y, than make correlation and significance test with the y’ and the y.

3. Results
3.1. The relationships between total chlorophyll content (Ct) and SPAD values of two different genotypes of poplars.
From the fig 1, we found (1) When the Ct value is the same, Populus simonii shows bigger SPAD than I-72, it tells we cannot treat the two different genotypes poplar as one type. (2) The linear relationship between Ct and SPAD is perfect when the SPAD is lower than 30 or more than 30, but not good within the scope of the entire. What is more, the SPAD value indicates relative Ct value. All in all, we cannot use SPAD value to predict the Ct value accurately.
3.2. The relationships between total chlorophyll content (Ct) and vegetation index of two different genotypes of poplars.

Using Ct of populus simonii, I-72 and the combined to fit linear, logarithmic (log) and exponential (exp) curve with the above vegetation indices, the results are shown in table 1. It is observed that (1) the exponential model of Ct and SDr/SDb index have best fitting effect of populus simonii, \( R^2 = 0.85 \); the exponential model of Ct and mSR705 index have best fitting effect of I-72, \( R^2 = 0.90 \); the logarithmic model of REPIG index have best fitting effect of the combined two kinds poplars, \( R^2 = 0.77 \).

(2) We find the three biggest \( R^2 \) in all the models, and they are REPIG, mSR705 and SDr/SDb. Meanwhile, we find the two smallest \( R^2 \) in all the models, and they are PSSRb and PRI.

![Fig 1. The Ct and SPAD distribution map of two different Genotypes](image)

Table 1. The fitting results \( R^2 \) of the total chlorophyll and vegetation indices

|       | populus simonii | I-72 | populus simonii and I-72 |
|-------|----------------|------|--------------------------|
| R²    | linear | log  | exp | linear | log  | exp | linear | log  | exp |
| CI    | 0.84** | 0.78** | 0.80** | 0.87** | 0.82** | 0.72** | 0.69** | 0.69** |
| PSSRb | 0.76** | 0.73** | 0.72** | 0.71** | 0.68** | 0.68** | 0.36** | 0.38** | 0.40** |
| mSR705| 0.83** | 0.76** | 0.83** | 0.87** | 0.76** | 0.90** | 0.75** | 0.70** | 0.76** |
| ND705 | 0.82** | 0.78** | 0.76** | 0.88** | 0.84** | 0.82** | 0.68** | 0.68** | 0.65** |
| SR    | 0.81** | 0.78** | 0.83** | 0.87** | 0.86** | 0.88** | 0.70** | 0.72** | 0.71** |
| PRI   | 0.49** | -     | 0.39** | 0.52** | -     | 0.36** | 0.45** | -     |
| RVI   | 0.84** | 0.76** | 0.84** | 0.83** | 0.71** | 0.86** | 0.59** | 0.55** | 0.63** |
| GMI   | 0.84** | 0.76** | 0.84** | 0.83** | 0.71** | 0.85** | 0.60** | 0.56** | 0.64** |
| VOG3  | 0.84** | 0.77** | -     | 0.85** | 0.74** | -     | 0.72** | 0.67** | -     |
| ZM    | 0.83** | 0.77** | 0.83** | 0.88** | 0.79** | 0.89** | 0.72** | 0.68** | 0.73** |
| SDr/SDb| 0.84** | 0.75** | 0.85** | 0.81** | 0.67** | 0.88** | 0.71** | 0.63** | 0.75** |
| REPIG | 0.79** | 0.76** | 0.79** | 0.88** | 0.89** | 0.88** | 0.75** | 0.77** | 0.75** |

* ** mean (double side) significantly related at the level of 0.01, * mean (double side) significantly related at the level of 0.05.

It is observed from fig 2 that (1) almost all the indices of the I-72 and populus simonii change with the Ct obviously. (2) Generally, the SR, PRI, VOG3 and SDr/SDb index of populus simonii is less than I-72 poplar, while the other 9 indices value of populus simonii are all bigger than I-72.
4. Discussion
We cannot use SPAD value to predict the Ct value accurately at all level, because it shows saturation causing by leaf structure and other circumstance as well as it only express relative content. The correlation of the two poplar combined Ct and 12 vegetation indices are all significant, we can choose proper vegetation indices to predict the Ct value, such as REPIG, mSR705 and SDr/SDb et al, their $R^2$ is relative higher. Meanwhile, two genotypes of poplars showed a certain difference on the spectral index, for example PSSRb and PRI et al., of which PRI was proved to be able to reflect differences between different genotypes of rice species [24]. Thus, we can find fine difference using PSSRb and PRI hyperspectral index if necessary.

5. Conclusion
This paper studied the relationships between Ct and SPAD and the 12 vegetation indices of two different genotypes of poplars. The results are as follows: (1) the exponential model of Ct and SDr/SDb index have best fitting effect of populus simonii, $R^2 = 0.85$; the exponential model of Ct and mSR705
index have best fitting effect of I-72, $R^2 = 0.90$; the exponential of Ct and mSR705 index have best fitting effect of the combined two kinds of poplars, $R^2 = 0.77$. (2) Almost all the indices of the I-72 and populus simonii change with the Ct obviously, and showed a consistency rule, which suggests that these spectral indices of different genotypes poplars changes similarly. (3) The SR, PRI and VOG3 index of populus simonii are little less than I-72 poplar, and the other 9 indices value of populus simonii are all little bigger than I-72, but the correlation of the two poplar combined Ct and 12 vegetation indices are all significant. That is to say, we can ignore genotypes to a certain extent, we also can distinguish genotypes using hyperspectral measurements when applying in Precision agriculture and other similar research filed.

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