The change of SPAD value and the establishment of correlation model of lettuce in intercropping mode

Linlin Wang[1,2,*], Tingting Song[1], Yuejiao Du[1], Que Zheng[1], Guangyu Zou[1]

[1]School of Science, Changchun Institute of Technology, 130012 Changchun, Jilin, China
[2]Research Center for Inorganic Chemistry, Changchun Institute of Technology, 130012 Changchun, Jilin, China

Abstract. In order to find out the change rule of SPAD value in lettuce leaves and the rapid, non-destructive and more accurate detection method of chlorophyll content in lettuce leaves under intercropping mode, the effect of intercropping on SPAD value in lettuce leaves was studied and the correlation model between chlorophyll content and SPAD value was established. The results show that the SPAD value of lettuce leaves increases at first and then decreases slowly with the development of the days of aerosol culture, and intercropping has obvious advantage in the middle and late stages of aerosol culture. At the same time, the results show that the regression equations of chlorophyll a, chlorophyll b and total chlorophyll content with SPAD value are best fitted by power equation, exponential equation and power equation respectively, and the coefficients of determination are 0.8302,0.6719 and 0.8503 respectively. The models are verified. It is found that the predicted value and the measured value of chlorophyll a, chlorophyll b and total chlorophyll content have a good correlation, and there is no significant difference, which shows that the regression models have a good prediction effect on chlorophyll content. The results provide a theoretical basis for the effect of intercropping on SPAD value of lettuce leaves and provide a reference method for rapid, non-destructive and more accurate detection of chlorophyll content in lettuce leaves.

1. Introduction

Photosynthesis is the main physiological basis of crop yield formation, and improving the utilization rate of light energy is an important way to achieve high yield of crops. The utilization rate of light energy in plants is very low, and improving photosynthetic performance is the fundamental measure to improve the utilization rate of light energy. The results showed that the measures such as selecting good varieties, improving cultivation management, suitable planting system and reasonable close planting could effectively improve the utilization ratio of light energy of crops[1]. Intercropping, as the essence of traditional agriculture, can increase the yield and income by increasing the area and time of photosynthesis. Chlorophyll is the main pigment which absorbs, transmits and converts light energy in the process of photosynthesis[2]. It is the necessary condition of photosynthesis and an important index of plant growth.

At present, there are two widely used methods for measuring chlorophyll, Spectrophotometer[3-4] and chlorophyll meter[5-6]. The Spectrophotometer measurements are more accurate and yield specific amounts of chlorophyll a, chlorophyll b and total chlorophyll, but they are time-consuming and can cause damage to the leaves. The chlorophyll meter works by emitting red and infrared light from two light-emitting diode towards a part of the leaf, and measuring the relative chlorophyll content (SPAD) using differences in optical density at two wavelengths[7]. It has the advantages of rapid measurement, simple operation, real-time data and non-destructive samples, but it also has its own limitations. The SPAD value measured by the instrument can only reflect the relative content of chlorophyll and experiments with precise data requirements can not be met. Moreover, the models of chlorophyll content and SPAD value of different plants are different, and the unified model between them is lacking[8].

In recent years, there are many reports about promotion of intercropping on SPAD value of crops[9-12] and the correlation between chlorophyll content and SPAD value[13-17], which provides a theoretical basis for predicting chlorophyll content by SPAD value. However, there is no report on the study of lettuce leaves in intercropping system. Therefore, the lettuce leaves were used as the research object under the model of aerated cultivation in intercropping between lettuce and cherry radish, the relationship between SPAD value and chlorophyll a, chlorophyll b and total chlorophyll content in lettuce leaves was studied, and the regression equation was established, in order to predict the chlorophyll content quickly, nondestructively and accurately, reflect the growth status of plants, and provide some reference for production practice.

* Corresponding author: 785181537@qq.com

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2. Materials and methods

2.1. Summary of the test site

The experiment was carried out in the research center of Inorganic Chemistry, Changchun Institute of Technology. During the experiment, the temperature was (25 ± 2) ° C at noon and (16 ± 2) ° C at evening. The full-spectrum plant supplementary light of Boyu biotechnology is installed indoors to control the light intensity at 5.5~6.5 klx, and the effect of supplementary light is shown in Fig. 1.

Fig. 1. Effect of supplementary light

2.2. Test materials and design

Italian lettuce and cherry radish were selected as experimental materials, and the seeds were purchased from Kefeng Seed Industry Co. , Ltd. After accelerating germination, the seeds were nurtured, transplanted when lettuce and cherry radish grew 3-4 functional leaves, and planted in aerosol cultivation boxes. The aerosol cultivation box is shown in Fig. 2. The lettuce and cherry radish were intercropped at a ratio of 1:1, at the same time, lettuce monoculture mode was set. Intercropping and monoculture mode are shown in Fig. 3. In the nutrient solution formula, the bulk element formula adopts the Japanese garden trial formula, and the micronutrient formula adopts the Hollander universal formula.

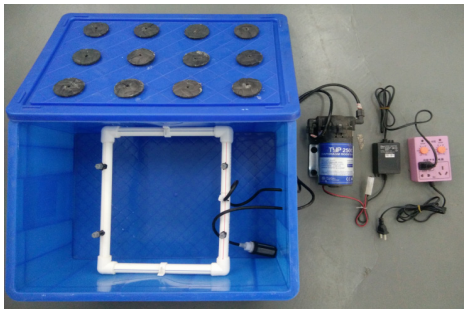

Fig. 2. Aerosol cultivation box

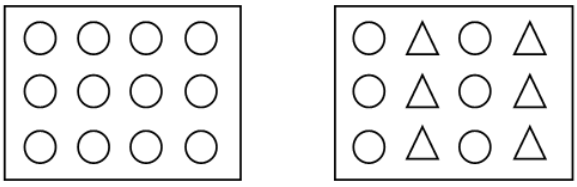

Fig. 3. Monoculture and intercropping mode

The SPAD value of three random lettuce leaves in intercropping and monoculture mode was measured on the 10th, 20th and 30th day after planting respectively. The data was used to study the effect of intercropping on SPAD value. After 30 days in aerosol culture, 40 middle leaves of lettuce plants were selected randomly and numbered, then SPAD value and chlorophyll content were determined respectively. The data was used to study the correlation between chlorophyll content and SPAD value.

2.3. Indicators and methods of measurement

Determination of SPAD value: the SPAD value of leaves was measured by FK-YL04 chlorophyll analyzer, and the average value was measured at 3 points of each leaf that avoided the vein, and the data were recorded according to the number. When the SPAD value was determined, the leaves did not need to be harvested in vitro.

Determination of chlorophyll content: after measuring the SPAD value of leaves, the chlorophyll content was determined by 95% ethanol extraction method[3]. After pre-treatment of fresh plant leaves, they were ground with quartz sand and ethanol until the tissues turned white, and then filtered and rinsed repeatedly to obtain the chlorophyll extract. Finally, with 95% ethanol as blank, the absorbance of chlorophyll extract was determined at 665 nm and 649 nm of the maximum absorption peaks of chlorophyll a and chlorophyll b, respectively.

2.4. Data collation and analysis

Microsoft Excel 2007 was used for data processing and calculation and SPSS 21.0 was used for significance test, correlation analysis, analysis of variance and paired sample t test.

The calculation formulas of chlorophyll a, chlorophyll b and total chlorophyll content are as follows:

$$C_a = (13.95A_{665} - 6.88A_{649})V / 1000W \quad (1)$$

$$C_b = (24.96A_{649} - 7.32A_{665})V / 1000W \quad (2)$$

$$C_t = (6.63A_{665} - 18.08A_{649})V / 1000W \quad (3)$$

Where, C_a is chlorophyll a content (mg/g), C_b is chlorophyll b content (mg/g), C_t is total chlorophyll content (mg/g), A_{649} is the absorbance of chlorophyll extract at 649 nm, A_{665} is the absorbance of chlorophyll extract at 665 nm, V is the volume of the chlorophyll extract (mL), W is fresh weight of the sample (g).

3. Results and analysis

3.1. Effect of intercropping on SPAD value

The SPAD value of lettuce leaves was measured on the 10th, 20th and 30th day after colonization in monoculture and intercropping modes, and the

differences of SPAD value between the two modes were compared. The results are shown in Fig. 4. The results show that the SPAD value of lettuce leaves is not significantly different between monoculture and intercropping at 10 days. Compared with the monoculture mode, the SPAD value of lettuce leaves is increased by 7.26% and 6.75% in the intercropping mode at 20 and 30 days, respectively, and there is significant difference between the two modes. It can be seen that intercropping mode has obvious advantage in improving SPAD value of leaves.

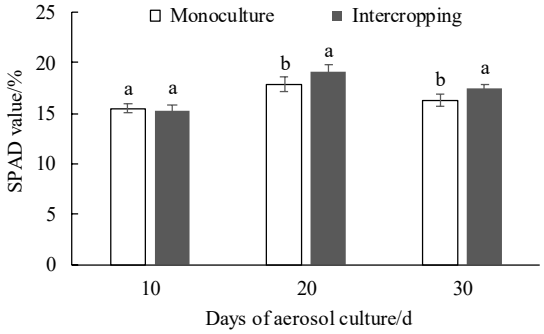

Fig. 4. Effect of intercropping on SPAD value

As a whole, the SPAD value of lettuce leaves increases at first and then decreases slowly with the advance of the days of aerosol culture. The reason is that the newly formed tissue of young leaves is not well developed, the lamellar structure of chloroplast is not developed, and the content of chlorophyll is less at 10 days of aerosol culture. With the growth of leaves, the synthesis rate of chlorophyll reaches the maximum value, so the maximum value of SPAD value of lettuce leaves appears at the time of 20 days, when the leaves are in the functional period of vigorous growth. After the functional period, the synthesis rate of chlorophyll decreases, which shows that the SPAD value of lettuce leaves has a slow decline trend at 30 days. Under this trend, the yield of lettuce will first have a rapid accumulation, then the growth and development of lettuce will slow, and the yield accumulation rate will decrease.

3.2. Establishment of correlation model between chlorophyll content and SPAD value

3.2.1 Results of chlorophyll content and SPAD value

The statistical results of chlorophyll content and SPAD value of 40 lettuce leaves which were selected are shown in Table 1. The data of 30 leaves were used to build the model and the data of 10 leaves were used to validate the model.

3.2.2 Correlation model between chlorophyll content and SPAD value

In order to obtain the optimal functional relationship between chlorophyll content and SPAD value, the linear equation, polynomial equation, power equation, exponential equation and logarithm equation of chlorophyll a, chlorophyll b and total chlorophyll content with SPAD value were established respectively, and the correlation analysis was carried out. The coefficient of determination R^2 is used to determine the model and the results are shown in Table 2.

Table 1. Statistical results of chlorophyll content and SPAD value of the samples

Index	Modeling				Validation			
	Maximum	Minimum	Mean	Standard deviation	Maximum	Minimum	Mean	Standard deviation
Chlorophyll a	0.3596	0.0893	0.2602	0.0743	0.3902	0.1055	0.2708	0.0806
Chlorophyll b	0.1188	0.0407	0.0778	0.0205	0.1078	0.0541	0.0832	0.0154
Total chlorophyll	0.4618	0.1309	0.3380	0.0912	0.4843	0.1596	0.3540	0.0929
SPAD value	16.20	6.90	12.85	2.17	16.80	9.23	13.40	2.11

Table 2. Correlation model between chlorophyll content and SPAD value

Type of equation	Chlorophyll a	Chlorophyll b	Total chlorophyll
Linear equation	y=0.0307x-0.1342 R^2=0.8007	y=0.0074x-0.0175 R^2=0.6113	y=0.0381x-0.1517 R^2=0.8198
Polynomial equation	y=-0.0005x^2+0.0432x-0.2059 R^2=0.8027	y=0.0003x^2+0.0002x+0.0241 R^2=0.6202	y=-0.0002x^2+0.0434x-0.1817 R^2=0.8200
Power equation	y=0.0029$x^{1.7530}$ R^2=0.8302	y=0.0038$x^{1.1783}$ R^2=0.6609	y=0.0056$x^{1.6004}$ R^2=0.8503
Exponential equation	y=0.0353e$^{0.1512x}$ R^2=0.7998	y=0.0196e$^{0.1044x}$ R^2=0.6719	y=0.0543e$^{0.1388x}$ R^2=0.8276
Logarithmic equation	y=0.3478ln(x)-0.6222 R^2=0.7941	y=0.0822ln(x)-0.1308 R^2=0.5811	y=0.4300ln(x)-0.7531 R^2=0.8064

The results show that in the regression equation between chlorophyll a content and SPAD value, the order of coefficient of determination from large to small is power equation, polynomial equation, linear equation, exponential equation and logarithmic equation, and the coefficient of determination of power equation is 0.8302, which shows that the fitting effect is good. In the regression equation of chlorophyll b content and SPAD value, the order of coefficient of determination from large to small is exponential equation, power equation, polynomial equation, linear equation and logarithmic equation, and the coefficient of determination of the exponential equation is 0.6719, which shows that the fitting effect is good. In the regression equation of total chlorophyll content and SPAD value, the order of coefficient of determination from large to small is power equation, exponential equation, polynomial equation, linear equation and logarithmic equation, the coefficient of determination of power equation is 0.8503, which shows that the fitting effect is good. Through the analysis of variance, the correlation of the above models has reached a very significant level.

3.2.3 Validation of correlation model between chlorophyll content and SPAD value

Using 10 samples as the test set of regression equation, the chlorophyll a, chlorophyll b and total chlorophyll content of lettuce leaves were predicted by using the regression model with good fitting effect. *T*-test was used to compare the predicted values with the measured values of Spectrophotometer. The results are shown in Table 3. As can be seen from Table 3, the coefficients of determination between predicted and measured values of chlorophyll a, chlorophyll b and total chlorophyll content are 0.7726, 0.6068 and 0.8046, respectively, and the *P* values are 0.5480, 0.5620 and 0.6390, which are much higher than 0.05. Therefore, the correlation between the predicted and measured values is good and the difference is not significant. The corresponding regression models can be used to predict the chlorophyll content of lettuce leaves under the intercropping mode.

Table 3. Comparison of predicted and measured value of chlorophyll content

Index	Standard deviation	Standard error	Coefficient of determination	*T* value	Degree of freedom	*P* value
Chlorophyll a	0.0385	0.0122	0.7726	-0.6240	9	0.5480
Chlorophyll b	0.0110	0.0035	0.6068	0.6030	9	0.5620
Total chlorophyll	0.0414	0.0131	0.8046	-0.4860	9	0.6390

4 Discussion and conclusion

4.1. Discussion

In the middle and late stages of lettuce growth, the SPAD value of lettuce leaves under the intercropping mode is significantly increased compared with monoculture, which is consistent with the results of previous studies on intercropping[18] . The reason may be that the above-ground growth space of cherry radish is smaller than that of lettuce, which is beneficial to the extension and growth of lettuce leaves. The chloroplast lamellar structure is well developed and the chlorophyll content is high. The positive correlation between SPAD value and chlorophyll content has been verified in many crops[7,13-17], and the change of SPAD value is consistent with the change of chlorophyll content.

There are differences in the better regression model and fitting correlation degree between chlorophyll content and SPAD value among different crops, even among different varieties of the same crop. Therefore, it is necessary to establish corresponding correlation models for different varieties to realize rapid diagnosis of crop growth status. In this study, the correlation between chlorophyll b and SPAD value is lower than that between chlorophyll a, total chlorophyll and SPAD value. This is related to the fact that 660nm which is the fixed wavelength when SPAD value is measured by the chlorophyll analyzer is very close to 665nm which is the absorption peak of chlorophyll a.

Chlorophyll content is closely related to photosynthesis, and leaf temperature and net photosynthetic rate are significantly positively correlated within the optimum temperature range for plant photosynthesis[19-20]. It has also been shown that there is a significant correlation between leaf temperature and light utilization efficiency[21]. Besides, the SPAD value can be obtained by the chlorophyll analyzer, and the relevant data of leaf temperature can also be obtained. In conclusion, the leaf temperature can be considered as another factor to build the model, but the fitting effect needs to be further studied.

4.2. Conclusions

The SPAD value of lettuce leaves increases at first and then decreases slowly with the development of the days of aerosol culture. The intercropping shows no advantage in increasing the SPAD value of lettuce leaves at 10 days, but has obvious advantage at 20 and 30 days. The results provide a theoretical basis for the effect of intercropping on SPAD value of lettuce leaves.

The better regression equations of chlorophyll a, chlorophyll b and total chlorophyll content with SPAD value are power equation, exponential equation and power equation respectively. After verification, the model's prediction effect is good, so the corresponding regression model can be used to predict the chlorophyll content. This provides a reference method for rapid, non-

destructive and more accurate detection of chlorophyll content in lettuce leaves under intercropping mode.

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