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# RELATIONSHIP BETWEEN RGB COMPONENTS AND PIGMENT CONTENTS IN RICE LEAF

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## Abstract

An attempt was taken to establish a methematical model for measuring the relation between RGB components of rice leaf colors and the content changes of various pigments. For this purpose the pigment content values of the leaves at different leaf positions and the values of their image RGB components at each growth stage of rice were studied. Besides curve fitting for describing the relationship between these two types of the values were adopted. The curves showed that the largest component value of the RGB was G; the second was R; and B was the smallest. Their mathematic models can perfectly describe the changes of leaf colors in rice. This study demonstrates that it is a approximate linear correlation between RGB components of rice leaf and its chlorophyll contents, facilitating the modelling and simulation of color changes of rice leaf.

## Introduction

With the development of inter-discipline approach between computer science and agriculture recently, the research on information technologies of crop growth has inspired many scholars (Cao *et al.* 2006, Schnable and Springer 2013, Gai *et al.* 2015). Rice researchers made lots of researches on the relation between rice leaf colors and nitrogen levels (Wang *et al*. 2002, Zhao *et al*. 2006, Jiang *et al*. 2012, Tang *et al*. 2014), which provide a theoretical basis for the nitrogen nutrition diagnosis of rice and reasonable nitrogen application. With the development of digital agriculture, scientists on crop modelling studied changes of leaf color to create virtual rice growth models. Through obtaining the SPAD values of leaves at different rice growth stages, the dynamic changes of leaf color at different leaf positions of rice stems to establish color simulation models of rice leaf based on SPAD values (Chang *et al.* 2007, Zhu *et al.* 2008, Wang *et al.* 2010, Vollmann *et al.* 2011, Yi *et al.* 2016a).

The change of leaf color is one of the important physiological indexes for reflecting the abundance or deficiency of nutrition in rice growth process, thus it becomes an indicator of rice cultivation. As the research data reveal, leaf colors of plant are related with the contained pigments in leaves, and proportions of the various pigment contents are a key of the color composition. The contained pigments in rice leaf mainly include chlorophyll a, chlorophyll b and carotenoid (xanthophyll and carotene), where chlorophyll a presents blue-green; chlorophyll b presents yellow-green; and carotenoid presents yellow (Feng 2007, Zhu *et al.* 2009, Wang *et al.* 2016). When rice reaches at the tillering stage, the sum of chlorophyll a and chlorophyll b in leaves is far more than carotenoid, making the leaves green. At this stage, the photosynthesis of leaves is strong, which is conductive for the rice growth. When the external environmental conditions such as temperature, moisture, nutrition, and other factors are changed or the rice enters the mature stage, the leaf pigment contents and their proportions will be updated. The degradation speed of chlorophyll a is higher than chlorophyll b, and the leaves gradually turn yellow. Meanwhile, while

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the total chlorophyll declines, content of the carotenoid increases, making the leaves yellow. Therefore, it may be concluded that application of the single SPAD value for describing rice leaf color is insufficient, for SPAD value cannot completely express the changes of pigment content of rice leaves during different growth stages, while pigment analysis complies with the inner color change rules of plant leaves.

To study the dynamic changes of rice leaf colors during different growth stages, this paper establishes a mathematical model for measuring the relation between RGB components of rice leaf colors and the content changes of various pigments. Furthermore, the 3D visualization technology is employed to inverse the dynamic changes of rice leaf colors during rice growth process on the computer, so as to provide decision-making assistance for rice researchers who study rice growth and regulation of fertilizer and water. As computer display system adopts tri-color model of R (red), G (green), and B (blue), which can express any color by mixing the three primary colors of red, green and blue in different proportions. With the color matching equation (1), the colors on the color display screen are all mixed by the three primary colors (Russ 2016). Since the leaf color depends on the contents of pigments in leaves, a corresponding pigment equation for RGB of leaf color component must be established to provide a basis for the dynamic simulation and visualization of rice leaf colors.

$$C = aR + bG + cB \qquad (1)$$

where $C$ is a matched color; the symbols R, G and B represent the red, green and blue colors; the coefficients $a$, $b$ and $c$ are the corresponding color values, respectively; and the symbol = means that the equation is a visual equivalent.

**Materials and Methods**

In this experiment two varieties of indica three-line hybrid early rice “Jinyou 458 (jy458)” and conventional early rice “Zhongjiazao 35 (zjz35)” were selected in different leaf colors as the experimental samples, of which the leaves of zjz35 were in dark green with 110 days as the whole growth period. The cultivation tests were conducted in the experimental station of Jiangxi Agricultural University (JXAU) successively in 2014 and 2015. On April 28, the rice was transplanted to the field for planting under the natural illumination with a light transmittance of 100%. To investigate the influences of different nitrogen levels on the leaf color changes, the experiment sets four nitrogen levels, which were N0 (0 g/mu), N1 (108 g/mu), N2 (216 g/mu) and N3 (324 g/mu), and thus there were 8 experiment zones (soil total nitrogen: 0.80 g/kg) for two varieties. Each nitrogen level was set for repeating four times, of which the average of the three repeated data was applied to modelling data and the fourth repeated data were utilized for the model tests. The fertilization patterns were divided into base fertilizers and topdressing, of which base fertilizers were applied (60%) during rice transplanting while the topdressing was applied (40%) during rice tillering. Other cultivation technologies were similar to the high-yielding rice cultivation technology, and meteorological data were provided by the agricultural meteorological station of JXAU.

For each leaf, it will acquire various parameters, including chlorophyll a (chla), chlorophyll b (chlb), carotenoid, SPAD, RGB-values of image and geometrical shape of the leaf. The test started after the samples of rice experiments were transplanted till the seedling stage. In order not to affect the natural growth of rice, the sampling period was around 6 days. The observation methods were as follows: firstly, selection of 4 plants with the similar growth vigor from each zone; secondly, Microtek color scanner (model: 1660XL Plus) scanning the pressure side of blades to obtain their image data with the scan resolution of 600 dpi; thirdly, the values of RGB-components of leaf images were extracted through VC++ programming; fourthly, the leaves were cut up and soaked in 95% ethyl alcohol until 72 hrs after the chlorophyll was all dissolved in ethyl alcohol to form chlorophyll extracting solution; finally, 722 (N) visible spectrophotometer was used to measure the absorbance of the chlorophyll a, b and carotenoid at the wave lengths of 665, 649 and 470 nm (Hartmut and Alan 1983).

*Calculation of various pigment concentrations in leaves:* The calculation formulas of various pigment concentrations in leaves extracted by ethanol soaking method are as equations (Hartmut and Alan 1983) (2) ~ (5).

$$C_a = 13.95D_{665} - 6.88D_{649} \quad (2)$$

$$C_b = 24.96D_{649} - 7.32D_{665} \quad (3)$$

$$C_x = (1000D_{470} - 2.05C_a - 114.8C_b) / 245 \quad (4)$$

$$C_{Total\ Chl\ a,b} = C_a + C_b = 18.08D_{649} + 6.63D_{665} \quad (5)$$

where $D_{665}$, $D_{649}$, and $D_{470}$ denote the absorbance of the chlorophyll a, b and carotenoid; $C_a$, $C_b$ and $C_x$ represent their concentrations; $C_{Total\ Chl\ a,b}$ refers to the total concentration of chlorophyll a and b; with the unit of mg/l for all parameters.

*Extraction of RGB values through programming:* The Photoshop software is utilized to remove the impurities in leaf images. Under the programming software environment of visual studio 2008, the color component values of R (red), G (green) and B (blue) in leaf images are extracted through the VC++ language programmed codes (Yi *et al.* 2016b).

## Results and Discussion

*Changes of rice leaf pigment contents at different growth stages:* The two sample varieties of jy458 and zjz35 were observed for pigment data of the main stem leaf under the medium nitrogen level. As shown in Table 1, TEP is the effective accumulative temperature; $C_T$ (chla+chlb) refers to the total chlorophyll; and $C_X$ signifies as the carotenoid. The curves are drawn as Fig. 1 according to the data in Table 1.

**Table 1. The changes of chlorophyll and carotenoid content at the different date.**

| Date (mm/DD/yyyy) | TEP (°C) | $C_T$_jy (mg/l) | $C_T$_zjz (mg/l) | $C_{X\_jy}$ (mg/l) | $C_X$_zjz (mg/l) |
|---|---|---|---|---|---|
| 05/13/2015 | 8 | 4.4 | 5.9 | 1.4 | 1.5 |
| 05/19/2015 | 83 | 7.7 | 7.6 | 1.2 | 1.0 |
| 05/25/2015 | 168 | 8.3 | 8.5 | 1.4 | 1.1 |
| 05/29/2015 | 237 | 8.7 | 8.0 | 1.2 | 1.4 |
| 06/04/2015 | 321 | 8.8 | 8.6 | 1.2 | 1.2 |
| 06/10/2015 | 404 | 8.5 | 8.7 | 1.4 | 2.1 |
| 06/16/2015 | 504 | 9.9 | 10.5 | 1.3 | 1.6 |
| 06/22/2015 | 596 | 10.2 | 10.6 | 1.2 | 1.2 |
| 06/26/2015 | 676 | 10.9. | 9.6 | 1.2 | 1.5 |
| 06/30/2015 | 766 | 9.7 | 7.6 | 1.3 | 0.6 |

The changes of total chlorophyll content and carotenoid with the effective accumulated temperature are illustrated in Fig. 1a,b, respectively. Their curves are divided into four stages: I:

the tillering stage; II: the jointing and young panicle differentiation stage; III: the heading and filling stage; and IV: the mature stage (in the late filling stage).

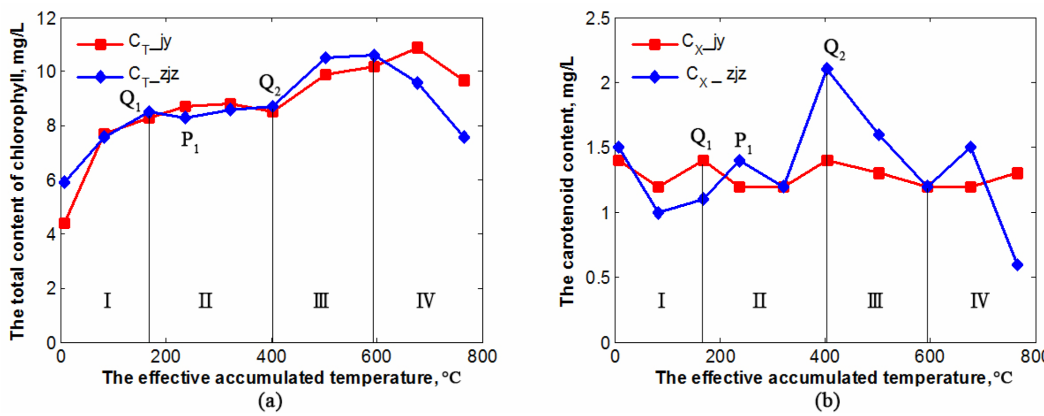


Fig. 1. Chlorophyll and carotenoid contents and their changes at different stages.

At the first stage, the chlorophyll contents increased with the rise of the effective accumulative temperature, while the carotenoid showed a declining trend, making the leaf color green (with a little black). When there was a certain nitrogen level at the tillering stage, the environmental temperature is the major factor affecting the chlorophyll biosynthesis. The second stage was the jointing and young panicle differentiation stage (during the late May to the early June), which is the overlapping stage of vegetative and reproductive growth of rice. During its turning period in growth, numerous reserves in leaves transported to growth cones of stems, and leaf color turned from dark green to light green; moreover, chlorophyll content decreased gradually with the increase of effective accumulated temperature, and leaf color became yellow. Due to the properties of zjz35 including dark green, tall and straight leaves, the change in leaf color was obvious with the decrease of chlorophyll content. As shown in Fig. 1(a), $Q_1$ rapidly reduced to the lowest point of $P_1$ and then slowly increased to $Q_2$. As the leaf color of zjz35 variety was dark green, the change of leaf color was also more obvious. The color change of both varieties was obvious at this stage of the overlap duration of vegetative growth and reproductive growth. In the growth transition period, most leaf nutrients were transported to the main stem, which resulted in the lightening of leaf colors, the reduction of chlorophyll, making the leaf color “yellow”. At the third stage, the chlorophyll content increased significantly, which improved the photosynthetic efficiency to produce more nutrition for satisfying the needs of rice booting, heading and filling while enhancing the maturing rate and rice yield. The chlorophyll content reached the maximum, and the leaf color presents “black”. Meanwhile, carotenoid showed a declining trend. The fourth stage from the late June to the middle July, the chlorophyll content gradually decreased, making the leaf color “yellow”. At this stage, the leaf nutrients were transferred to rice for the filling, and the rice mainly depends on flag leaves for photosynthesis. Hence, this stage is also a process for leaf chlorophyll to gradually fade. Fig. 1(b) is the curves of the carotenoid with the changes of the effective accumulative temperature, which corresponds to Fig. 1(a). The curve in Fig. 1(a) showed a rise while that of Fig. 1(b) a decline.

From the above analysis, it is apparent that the changes of chlorophyll content at different growth stages make the leaf color to present the physiological phenomenon of alternate variations between “black-yellow”.

*The relationship between RGB-values of color and total chlorophyll contents of rice leaf:* On the MATLAB R2013a platform, the fitting relationships between RGB components and total chlorophyll content of main-stem leaves of the plants were constructed and presented in Fig. 2.

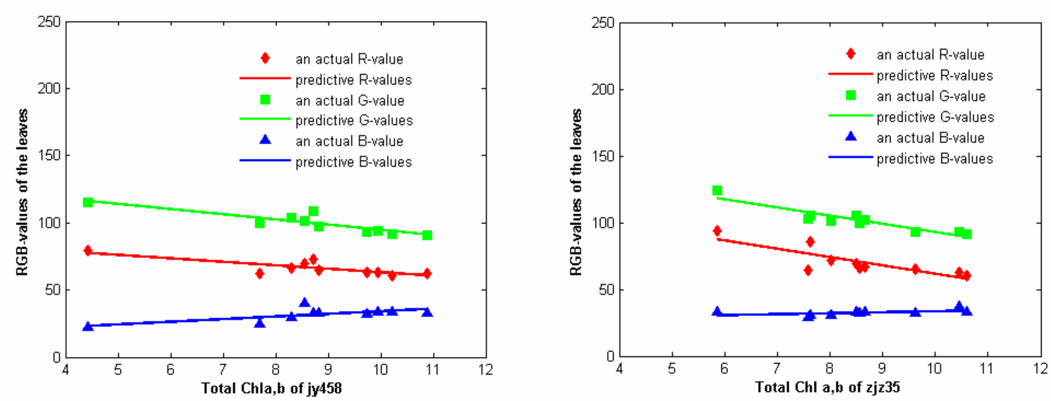


Fig. 2. Dynamic changes of RGB-components and total chlorophyll contents in leaves of different rice varieties.

In addition, the mathematical formulas and the correlation coefficients of the above relationships were obtained, as shown in equations (6) ~ (11).

- The variety of jy458

$$R_{jy458} = -2.5843C_T + 88.961, \quad (r^2 = 0.6336) \tag{6}$$

$$G_{jy458} = -3.8512C_T + 133.2206, \quad (r^2 = 0.7743) \tag{7}$$

$$B_{jy458} = 1.961CT + 14.481, \quad (r^2 = 0.4969) \tag{8}$$

- The variety of zjz35

$$R_{zjz35} = -6.24C_T + 123.9433, \quad (r^2 = 0.6656) \tag{9}$$

$$G_{zjz35} = -6.1231C_T + 154.0442, \quad (r^2 = 0.8666) \tag{10}$$

$$B_{zjz35} = 0.7279C_T + 26.177, \quad (r^2 = 0.252) \tag{11}$$

As the fitted curves in Fig. 2 and the obtained equations reveal, the RGB change of two rice varieties along with the changes of chlorophyll, where the green component is larger than the red one, with the blue component the least. In the equations (6) ~ (11), the fitting coefficient of each component is $r_G^2 > r_R^2 > r_B^2$, and thus the rice leaf color presents green.

*Model tests.* The simulation values of RGB can be obtained by substituting the obtained model test data into the above formulas. The internationally popular root mean square error (RMSE) is applied for calculating the simulation precision of RGB.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(OBS_i - SIM_i)^2}{n}} \tag{12}$$

where $OBS_i$ is an observed value or a measured value, and $SIM_i$ is a simulated, and $n$ is a number of the samples. As the results indicate, the simulation values were consistent with the observed values, proving the fine simulation fitting of the model. Meanwhile, their 1-1 relational graphs were drawn to test the model reliability, as shown in Fig. 3.

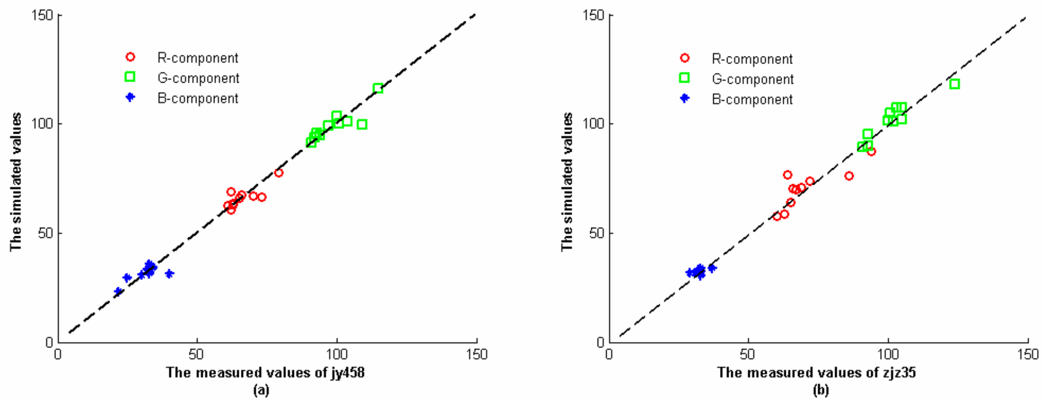


Fig. 3. The comparative results between observed and simulated values of the RGB.

*Regression analysis between RGB components and various pigments:* Multiple linear regression analysis (MLRA) is implemented in the simultaneously obtained the RGB component and leaf pigment values to establish the regression equations. MATLAB 2013a software was adopted to conduct regression analysis for concluding the MLR equations (13) ~ (18).

- Regression equations of jy458

$$R_{jy458} = 124.169 - 6.318C_a - 2.383C_b - 7.695C_x, \quad r^2 = 0.822 \tag{13}$$

$$G_{jy458} = 147.007 - 3.454C_a - 8.175C_b - 3.374C_x, \quad r^2 = 0.845 \tag{14}$$

$$B_{jy458} = 59.899 - 2.105C_a + 0.101C_b - 10.382C_x, \quad r^2 = 0.405 \tag{15}$$

- Regression equations of zjz 35

$$R_{zjz35} = 81.061 - 13.04C_a + 10.952C_b + 36.3C_x, \quad r^2 = 0.959 \tag{16}$$

$$G_{zjz35} = 140.502 - 7.663C_a - 2.932C_b + 12.427C_x, \quad r^2 = 0.906 \tag{17}$$

$$B_{zjz35} = 17.535 - 0.109C_a + 2.227C_b + 8.162C_x, \quad r^2 = 0.634 \tag{18}$$

where $C_a$, $C_b$ and $C_x$ represent the concentrations of chla, chlb and carotenoid with the unit of mg/l.

The analysis results further indicate, there are significant linear correlations between R and G components and pigment concentrations, while the correlation between B component and pigment concentration changes is insignificant. For the data of two groups, the linear correlation of zjz35 variety was higher than that of jy458 variety, as the leaf color of zjz35 was dark green, and the leaves were straight. Therefore, the relation between RGB and leaf pigments can be presented by linear multivariate equations, and the equation coefficients can be obtained through multiple regression analysis of test data.

*Relationship between RGB values and pigments at different leaf positions:* There were differences in leaf pigment contents at different leaf positions. By applying linear regression analysis method for observed data at different leaf positions, the relationship between RGB values and pigments at different leaf positions could be obtained, as shown in Tables 2 and 3.

**Table 2. Regression coefficients between RGB components and leaf pigments at the tillering stage.**

| | The $5^{th}$ leaf | | | The $6^{th}$ leaf | | | The $7^{th}$ leaf | | | The $8^{th}$ leaf | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *R* | *G* | *B* | *R* | *G* | *B* | *R* | *G* | *B* | *R* | *G* | *B* |
| jy458 | 0.762 | 0.922 | 0.290 | 0.636 | 0.563 | 0.680 | 0.814 | 0.666 | 0.456 | 0.999 | 0994 | 0.156 |
| zjz35 | 0.996 | 0.999 | 0.981 | 0.998 | 0.951 | 0.783 | 0.949 | 0.815 | 0.838 | 0.981 | 0.953 | 0.640 |

**Table 3. Regression coefficients between RGB components and leaf pigments at the jointing and young panicle differentiation stage.**

| | The 9th leaf | | | The 10th leaf | | | The 11th leaf | | | The 12th leaf | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | G | B | R | G | B | R | G | B | R | G | B |
| jy458 | 0.693 | 0.795 | 0.347 | 0.833 | 0.884 | 0.820 | 0.959 | 0.962 | 0.851 | 0.820 | 0.959 | 0.941 |
| zjz35 | 0.787 | 0.884 | 0.563 | 0.983 | 0.947 | 0.692 | 0.984 | 0.917 | 0.358 | 0.992 | 0.993 | 0.820 |

Tables 2 and 3 indicate that the G-component of the RGB is the largest while B-component is the smallest. As shown in the tillering stage of Table 2, the correlative coefficient of the 5th and 8th leaves is $r^2 > 0.9$, indicating G is high-positive correlated with chlorophyll, and the leaves were dark green. The 6th and 7th leaves were at the jointing stage, which was the initial overlap duration of vegetative growth and reproductive growth. At this period, the G-component of chlorophyll decreased; the R-component is large, but the B-component was still small, making the leaves light green, which was similar with the analysis results of the second stage. At the jointing and young panicle differentiation stage (Table 3), both G and R components were large, and B-component was still the smallest. The chlorophyll G-component of the $12^{th}$ leaf (flag leaf) was high, which suggests the photosynthesis ability with large chlorophyll content is strong enough to provide sufficient nutrition for the rice grain filling. For the same leaf position of different varieties, the G-component of zjz35 was significantly higher than that if jy458, highlighting the property of dark green color of zjz35. Through analysis, it may be suggested that the chlorophyll contents of different leaf positions are different, and the leaf colors are also different, showing the leaf color changing differences between different rice varieties.

There are a few reports on establishing the mathematical models for evaluating the relation between RGB values of leaf colors and various pigment contents with dynamic pigment content changes of rice leaf colors during different growth stages. Rice color not only is an important indicator for regulating fertilizers and water during growth, but also is closely associated with the fertilizer degree. Hence, researchers have carried out a lot of research on rice colors from perspectives of reasonable fertilizing and improving ecological environment. They usually selected the SPAD value as the rice color indicator to study the relationships between SPAD value and nitrogen absorption (Zhao *et al.* 2006), as well as between leaf color information of different leaf positions and leaf nitrogen contents (Tang *et al.* 2014). There are other reports on SPAD as color indicator of plant leaves, such as studying the wheat SPAD values and RGB (Zhu *et al.* 2008), and simulated models of dynamic rice leaf color changes (Chang *et al.* 2007), hoping to establish wheat and rice leaf color models based on SPAD values. The authors believe SPAD value can be utilized for qualitative analysis of rice leaf colors, but pigment analysis is needed for studying the dynamic changes of rice leaf colors during different growth stages. For instance, when rice leaves enter the aging stage, the speed of chlorophyll degradation is higher, and the lutein and carotenoid become the major impacts, making the leaves present yellow. Therefore, while studying rice leaf colors, pigment indicators like chla, chlb, and carotenoid can be adopted to characterize the leaf color changes. The mathematical model between RGB values and different leaf pigments established in this way also conforms to the principles of plant colors and rice leaf changing rules.

The rice leaf color changes at different growth stages need to be consistent with the growth rules, which is also the premise to establish the mathematical model between leaf color RGB and chla, chlb, and carotenoid. As is indicated in Fig. 1, from the seedling to the tillering stage, the chlorophyll content is more than carotenoid, and thus the leaf color is dark green. When at the jointing and young panicle differentiation stage, the chlorophyll content reduces while carotenoid content increases, making the leaf color light green. After entering the heading and filling stage, the chlorophyll content rises obviously, and the leaves present dark green again. At the later filling stage, the chlorophyll content drops again, making the leaf color gradually turn to light green or yellow. Such "black yellow" alternating phenomenon of rice leaf colors complies with the leaf color change rules during rice growth. Only establishing a mathematical model by studying the relationships between RGB of rice leaf colors and chla, chlb and carotenoid based on this can the model accurately express the dynamic changes of rice leaf colors.

In RGB components, G component is the mostly relative to leaf pigment. With MATLAB 2013a software as the analysis tool, this paper conducts multiple linear regression analysis for the correlation between RGB components and leaf pigments from two aspects of rice plants and each leaf position. As the analysis results indicate, the correlation coefficient $r^2$ of G-component is largest, followed by R-component, which is also revealed in equations (6) ~ (11) and (13) ~ (18). The correlation coefficient of each leaf position in Tables 2 and 3 verifies this conclusion again, which suggests the uniformity of the whole and the individuals. The regression equations (13) ~ (18) concluded by analyzing two experimental varieties can be taken as the theoretical basis for the simulation and visualization modelling of rice leaf colors.

The conclusions of this study are only targeted for the early rice varieties of mid Jiangxi areas in the middle and lower reaches of the Yangtze River, and further extension research is needed for middle-season and late rice as well as other regions.

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