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Research Article

A study on cotton yield prediction based on the chlorophyll fluorescence parameters of upper leaves

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Abstract

The early and accurate monitoring of crop yield is important for field management, storage needs, and cash flow budgeting. Traditional cotton yield measurement methods are time-consuming, labor-intensive, and subjective. Chlorophyll fluorescence signals originate from within the plant and have the advantages of being fast and non-destructive, and the relevant parameters can reflect the intrinsic physiological characteristics of the plant. Therefore, in this study, the top four functional leaves of cotton plants at the beginning of the flocculation stage were used to investigate the pattern of the response of chlorophyll fluorescence parameters (e.g., F0, Fm, Fv/F0, and Fv/Fm) to nitrogen, and the cumulative fluorescence parameters were constructed by combining them with the leaf area index to clarify the correlation between chlorophyll fluorescence parameters and cotton yield. Support vector machine regression (SVM), an artificial neural network (BP), and an XGBoost regression tree were used to establish a cotton yield prediction model. Chlorophyll fluorescence parameters showed the same performance as photosynthetic parameters, which decreased as leaf position decreased. It showed a trend of increasing and then decreasing with increasing N application level, reaching the maximum value at 240 kg·hm^{-2} of N application. The correlation between fluorescence parameters and yield in the first, second, and third leaves was significantly higher than that in the fourth leaf, and the correlation between fluorescence accumulation and yield in each leaf was significantly higher than that of the fluorescence parameters, with the best performance of Fv/Fm accumulation found in the second leaf. The correlation between Fv/Fm accumulation and yield in the top three leaves combined was significantly higher than that in the top four leaves. The correlation coefficient between Fv/Fm accumulation and yield was the highest, indicating the feasibility of applying chlorophyll fluorescence to estimate yield. Based on the machine learning algorithm used to construct a cotton yield prediction model, the estimation models of Fv/F0 accumulation and yield of the top two leaves combined as well as top three leaves combined were superior. The estimation model coefficient of determination of the top two leaves combined in the BP algorithm was the highest. In general, the Fv/F0 accumulation of the top two leaves combined could more reliably predict cotton yield, which could provide technical support for cotton growth monitoring and precision management.

Keywords: cotton; chlorophyll fluorescence parameters; leaf position; machine learning; yield

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Introduction

Cotton is one of the most important cash crops in the world; it is not only a major raw material for the textile industry, but also plays an important role in national defense, medicine, paper, and furniture industries (Xu *et al.*, 2021). In the agricultural production process, the early and accurate estimation of crop yield is pivotal for crop management, which not only helps processors to develop harvest demand plans and management to regulate import and export policies, but also helps farmers to make sound agronomic decisions, regarding, e.g., water and fertilizer inputs, crop insurance, harvest planning, and cash budgets at late reproductive stages (Ashapure *et al.*, 2020). Traditional methods of measuring yield information are extensive destructive sampling and manual surveys of cotton fields at harvest time, which require high amounts of time and labor and are highly subjective and time-sensitive (Pantazi *et al.*, 2016). Modern information technologies such as remote sensing and image recognition have the advantages of being non-destructive and rapid, and have been widely used in recent years for the agricultural monitoring of crop physiology and biochemistry, nutrient abundance and deficiencies, and adversity stress (Guo *et al.*, 2016; Gabriel *et al.*, 2017; Qu *et al.*, 2017; Zhao *et al.*, 2018). Previous studies have used canopy monitoring sensors to monitor the nutritional status and yield traits of cotton (Yang *et al.*, 2006; Ashapure *et al.*, 2020), wheat (Li *et al.*, 2014; Dehkordi *et al.*, 2020), maize (Fang *et al.*, 2011; Li *et al.*, 2014), and other crops. The results of these studies show that it is feasible to use canopy information to monitor crop growth and predict crop yield, but the obtained canopy information involves many factors such as leaves, stalks, abscissions, and soil, which leads to low accuracy and large errors in crop yield prediction.

Chlorophyll fluorescence signals originate from within the plant (Kalaji *et al.*, 2017). The relevant parameters can reflect the intrinsic physiological properties of the plant and have the advantages of rapid, non-destructive, and simple determination and have been successfully applied to plant health and growth condition monitoring (Ptushenko *et al.*, 2014). Previous studies have shown that chlorophyll fluorescence can be used to monitor the physiological changes of crops under high temperature, drought, pests, and other adversity conditions and can better reflect the resistance of crops to stress (Calatayud *et al.*, 2013). Under high temperature conditions, the maximum photochemical quantum efficiency Fv/Fm decreased significantly, while the minimum fluorescence F0 increased (Janka *et al.*, 2013); under drought conditions, the actual photochemical quantum yield ΦPSII of soybean seedling leaves was most correlated with the degree of drought (Wang *et al.*, 2018), and the fluorescence parameter Fv/Fm of olive leaves had a significant correlation (Faraloni *et al.*, 2010). In addition to adversity stress, chlorophyll fluorescence parameters can also better reflect the nutritional status of crops, and Feng *et al.* (2015) concluded that chlorophyll fluorescence parameters are ideal indicators for evaluating the nitrogen nutritional status of winter wheat. However, most of these studies focused on the changes of fluorescence parameters in crops under adversity and different nutrients, while relatively few studies have used chlorophyll fluorescence parameters to monitor and evaluate crop yield. Chlorophyll fluorescence is closely related to the physiological process of photosynthesis (Chaerle *et al.*, 2004) and is an important indicator for the evaluation of crop photosynthesis (Kalaji *et al.*, 2014). Photosynthesis is the basis of crop growth and yield formation, and is closely related to nutrient uptake and utilization by crops (Lin *et al.*, 2016). Crop yield mainly depends on the amount of photosynthetic product formation and accumulation during growth, and photosynthetic product accumulation is determined by the light energy conversion efficiency of photosynthesis, so it is feasible to apply chlorophyll fluorescence technology to monitor crop yield. Schachtl *et al.* (2005), by measuring chlorophyll fluorescence, found a strong correlation between fluorescence signals and aboveground dry matter yield, N concentration in dry matter, and N uptake in wheat canopies; Araus *et al.* (1998) showed that fluorescence parameters F0, Fm, and Fv were genetically correlated with wheat yield. Souza *et al.* (2022) screened five fluorescence indices based on fluorescence optical sensors to predict the N content and relative yield of pepper. The use of fluorescence information to invert crop yield status has been recognized to some extent, but most previous studies have focused on grain crops, vegetable crops, etc. Fewer studies have been conducted on cotton yield estimation, and most of them have selected a single leaf position, which limits the improvement of yield prediction accuracy. Therefore, the establishment of a cotton yield prediction method with high accuracy and good stability will be helpful for the agricultural macro-control and technical guidance of field management.

In this study, in order to provide theoretical and technical support for cotton yield prediction, we analyzed the relationship between chlorophyll fluorescence parameters and the yield of the top four functional leaves of cotton under different nitrogen application conditions, combined with the leaf area index, to construct fluorescence accumulation, and we then established a rapid prediction model of cotton yield based on this chlorophyll fluorescence accumulation.

Materials and Methods

Test area overview

The experiment was conducted in the second company of the teaching experiment site of Xinjiang Shihezi University (44°26.5′N, 86°01′E), which is an arid and semi-arid region with a temperate continental climate, an annual sunshine time of 2721-2818 h, an annual average temperature of 5 °C (≥°C), and an effective cumulative temperature of 3570-3729 °C. The soil in the test area was loamy, with a field water holding capacity of 30.6 g-kg^{-1}, a soil bulk weight of 1.53 g·kg^{-1}, a soil organic matter concentration of 19.90 g·kg^{-1}, an alkaline decomposition nitrogen concentration of 60.88 mg·kg^{-1}, an effective phosphorus concentration of 17.95 mg·kg^{-1}, and an effective potassium concentration of 134 mg·kg^{-1}, and the previous crop was cotton.

Figure 1. Study area overview map

Experimental design

The experiment was conducted in the 2019 cotton growing season, and the cotton variety tested was Xinlu Early 58, planted with drip irrigation under a film, with a cultivation pattern of 1 film, 3 tubes, and 6 rows, a film width of 2.05 m, a plant spacing configuration of 10 cm + 66 cm, and a planting density of 21×10^4 plants-hm^{-2}. Treatments contained 0 (N0), 120 (N1), 240 (N2), and 360 (N3) kg·hm^{-2} (Figure 2), where N0 was the control. The experiment was conducted in a one-way completely randomized group design with three replications and a total of 12 experimental plots, each with an area of 2.25 m^2 and an isolation zone between the plots. The trial was mulched and sown on April 24, 2020, and harvested on October 1, 2020. Fertilizer was applied by drip irrigation with water, with 30% of the nitrogen fertilizer applied to the soil as a base fertilizer before sowing and the remaining 70% used in six follow-up applications (Table 1). The irrigation amount was the general irrigation amount of local drip irrigation cotton fields. Other field management during the whole reproductive period was carried out according to the high-yielding cultivation technology of Xinjiang, and attention was paid to the prevention of pests, diseases, and weeds.

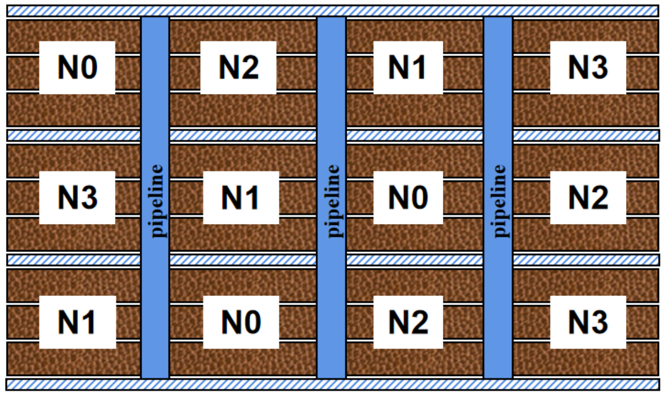

Figure 2. Study area plot layout map

Table 1. Date and rate of nitrogen application

Date (m-d)	Nitrogen Application Ratio/%
Before Sowing	Base Fertilizer 30
6-13	8
6-23	8
7-14	12
7-25	15
8-05	15
8-16	12

Measurement content and method

Determination of chlorophyll fluorescence parameters

The minimum fluorescence in light (F0'), the maximum fluorescence in light (Fm'), the initial fluorescence (F0), and the maximum fluorescence (Fm) of the upper four leaves of the cotton at different fertility periods were measured using a PAM-2100 portable pulse-modulated chlorophyll fluorometer. The chlorophyll fluorescence parameters were measured every 10 d during the period from flowering to flocculation, and the sampling times were 70 d (first flowering), 80 d (first flowering), 100 d (first boll), 110 d (first boll), 115 d (second boll), and 120 d (flocculation) after seedling emergence. The measurement site was the upper four fully expanded leaves of the cotton main stem, i.e., the top four leaves. Three cotton plants with uniform growth were randomly selected for each nitrogen treatment. The measurement was repeated three times, and the average value was calculated. The fluorescence parameters F0' and Fm' under light acclimation were measured at 12:00-16:00 Beijing time, and the fluorescence parameters F0 and Fm under dark acclimation were measured after the plants were fully dark-acclimated for at least 20 min during 22:30-24:00 that night. Variable fluorescence (Fv'), the maximum light energy conversion potential of PSII (Fv/F0), the potential maximum photochemical efficiency of PSII (Fv/Fm), and the maximum photochemical efficiency of PSII (Fv'/Fm') were calculated, respectively, as follows: Fv=Fm-F0; Fv'=Fm'-F0'; Fv/F0=(Fm-F0)/F0; Fv/Fm=(Fm-F0)/Fm; Fv'/Fm'=(Fm'-F0')/Fm'.

Yield composition factors and determination of yield

The measurement period was the beginning of flocculation. After the fluorescence parameters were measured, the total number of individual bolls of the cotton plant was measured, and the average single boll weight was calculated. The yield of seed cotton in the plot was calculated by recording the actual weight of 1 m^2 of cotton harvested in each plot, which included the measured plants.

Determination of leaf area index

After the determination of chlorophyll fluorescence parameters, the leaf area index of the top four main stem leaves was determined simultaneously. The leaf area of the top four leaves of a single cotton plant was measured using the LI-3100C benchtop leaf area meter from LI-COR, USA, and the leaf area index (LAI) was then calculated with the following formula: LAI = leaf area/land area.

Calculation of cumulative chlorophyll fluorescence parameters

Considering Fm as an example, for a single leaf, the Fm of the top leaf multiplied by the leaf area index of that leaf is its Fm accumulation. The Fm accumulation of the top two leaves, top three leaves, top four leaves, and other single leaves is calculated in the same way. The cumulative amount of two adjacent leaves, three adjacent leaves, and the top four leaves is the sum of the relevant cumulative amounts. The combination of the top two leaves is expressed as L12, that of the second and third leaves is expressed as L23, that of the top three leaves is expressed as L1-3, and that of the top four leaves is expressed as L1-4. The cumulative amount of each leaf position for other fluorescence parameters was calculated with the above algorithm determining the cumulative Fm amount.

Data processing and analysis

In this study, the data were simply processed using Microsoft Office Excel, and the chlorophyll fluorescence parameters, the plant population leaf area index, the number of bolls per plant, and the single boll weight were analyzed and plotted using SPSS-26.0 and R-4.1.3. A machine learning algorithm was then used to build a yield estimation model and evaluate the accuracy, and the research methods included a support vector machine, an artificial neural network, and XGBoost.

Support vector machines

A support vector machine (SVM) is a machine learning model that uses a hyperplane (or a line for two-dimensional data) and then vectorizes the data to maximize this segmentation. In this paper, we use the linear kernel function in SVM, which is defined as shown in Equation (1).

$$k(u, v) = u'v \quad (1)$$

Artificial neural network

An artificial neural network (ANN) is composed of many processing units and their connections, and the processing unit is the most basic unit of the neural network. The input and output equations of the processing unit are shown in Equation (2).

$$f_i = f(net_i) = f(\sum_i W_{ij} X_i - \theta_i) \quad (2)$$

where f_i is the output signal of the artificial neural network processing unit; net_i is the integration function; f is the transformation function of the artificial neural network processing unit; W_{ij} is the connection weight value among the processing units of the artificial neural network; X_i is the input vector; ϑ_i is the threshold value of the processing unit.

The most widely used artificial neural network architecture is the back propagation (BP) artificial neural network, which follows the basic principle of the gradient steepest descent method to minimize the error in an iterative manner. The model used in this paper is a BP neural network algorithm in an ANN.

Extreme gradient boosting

Extreme gradient boosting (XGBoost) is an optimized distributed gradient enhancement library that uses a combinatorial method of propulsion and a base learner of a regression tree. The objective function during training consists of two parts: the first part is the gradient boosting algorithm loss; the second part is the regularization term. The loss function is defined in Equation (3) as

$$L(\varphi) = \sum_{i=1}^{n} l(x_i, y_i) + \sum_{k} \Omega(f_k) \tag{3}$$

where x_i is the simulated value; y_i is the actual value, n is the number of samples available for validation, and $\sum_{i=1}^{n} l(x_i, y_i)$ indicates the prediction error of the ith sample, with lower error values indicating better model predictions. $\sum_k \Omega(f_k)$ is a function of the regularization term, which represents the complexity of the tree, and is defined as shown in Equation (4).

$$\Omega(f) = \gamma T + \frac{1}{2}\lambda\|w\|^2 \tag{4}$$

γ and λ are manually set parameters, w is the vector formed by the values of all leaf nodes of the decision tree, and T is the number of leaf nodes. A smaller regularization value indicates a lower complexity and an improved generalization ability.

Precision evaluation index

The accuracy of the yield estimation model was evaluated in terms of the coefficient of determination (R^2), the root mean square error (RMSE), and the standardized root mean square error (n-RMSE). The larger the R^2, the better the model fit, and the smaller the *RMSE* and n-RMSE, the smaller the deviation between the simulated and actual values, and the higher the model accuracy. The above indicators are calculated as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(x_i - \bar{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \tag{5}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i)^2}{n}} \tag{6}$$

$$n - RMSE = \frac{RMSE}{\bar{y}_i} \times 100\% \tag{7}$$

where x_i is the simulated value; y_i is the actual value; $\bar{y}_i$ is the average of the actual values; and n is the number of samples available for validation.

Results

Effect of the nitrogen application level and leaf position on leaf chlorophyll fluorescence parameters

The chlorophyll fluorescence parameter Fv/F0 can reflect the potential activity of Photosystem II, and Fv/Fm can reflect the original light energy conversion efficiency of Photosystem II. The larger the value of Fv/Fm, the lower the degree of the photoinhibition of plant leaves. As can be seen in Figure 3, the chlorophyll fluorescence parameter Fv/F0 of the top one leaf showed a gradual decrease under different nitrogen treatments from 70 to 120 d after seedling emergence, with the fastest decrease between 18.24% and 24.28% from 70 to 80 d after seedling emergence, followed by a slow decrease (from 80 to 120 d after seedling emergence). The analysis of fluorescence parameters Fv/F0 at different nitrogen levels showed that the maximum Fv/F0 was observed in the N2 treatment at different fertility periods, but the difference from other nitrogen treatments was not significant. The minimum Fv/F0 was observed in the N0 treatment, and the difference from other nitrogen treatments was significant from 70 to 115 d after seedling emergence. The top one leaf fluorescence parameter Fv/Fm showed the same pattern of change as Fv/F0 in general with the advancement of the fertility process, with the greatest decrease between 70 and 80 d after emergence, ranging from 3.00% to 4.19%. The greatest Fv/Fm under different fertility periods was exhibited under the N2 treatment, with, compared with the N0 treatment, an increase of 3.66% at 70 d after emergence and an increase of 3.66% at 120 d after emergence. At 70 d after seedling emergence, Fv/Fm increased by 3.66% and, at 120 d after seedling emergence, increased by 2.30%. Taken together, a moderate application of nitrogen increased the Photosystem II activity of the plant.

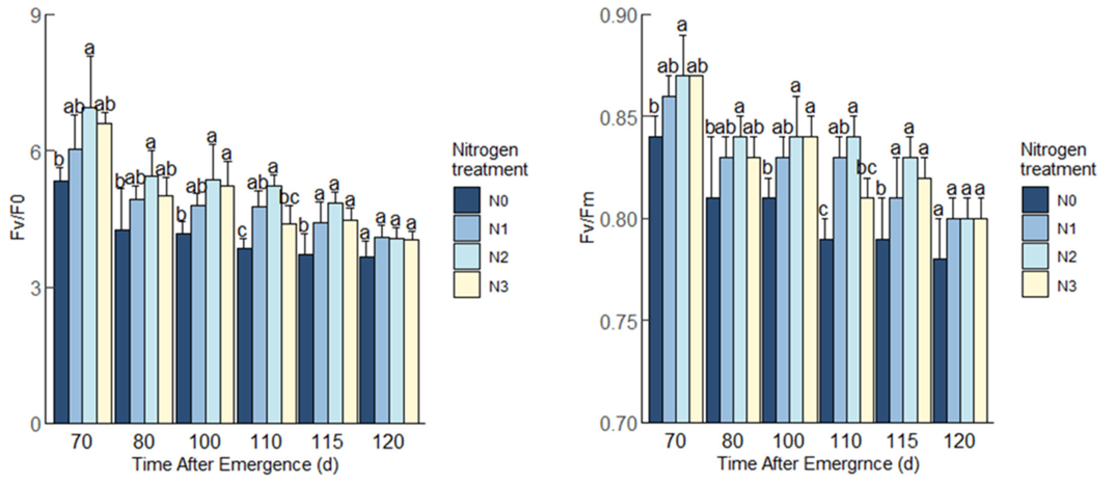

Figure 3. Dynamics of chlorophyll fluorescence parameters Fv/F0 and Fv/Fm in the cotton terminal leaf with the fertility process under different nitrogen levels

The effective photochemical quantum yield of PSII (Fv'/Fm') can reflect the primary light energy capture efficiency of the open Photosystem II reaction center. As can be seen in Figure 4, under nitrogen deficiency (N0) conditions, the chlorophyll fluorescence parameters Fv' and Fv'/Fm' of the top three leaves were not significantly different, while Fv' and Fv'/Fm' of the fourth leaf were significantly lower than the other leaves, and the decrease in Fv' and Fv'/Fm' ranged from 26.17% to 30.70% and 20.20% to 24.84%. With the improvement of N nutrition, the fluorescence parameters Fv' and Fv'/Fm' gradually increased and reached the maximum value under the N2 (240 kg·hm^{-2}) treatment, and the comparison between the N2 treatment and the N1 treatment showed that the increment of Fv' and Fv'/Fm' in the fourth tetrafoliate under the N2 treatment was greater than that of the top three leaves, increasing by 54.08% and 50.24%, respectively. Under the N0-N2 treatments, the differences in Fv' and Fv'/Fm' between the fourth leaf and the other three leaves were significant and all decreased slightly when the nitrogen application level was increased to N3 (360 kg·hm^{-2}), at which point the differences in Fv' and Fv'/Fm' were not significant. This indicates that a moderate application of nitrogen is beneficial to improve leaf light energy utilization, while both nitrogen deficiency and excessive application reduce the light energy conversion rate and affect photosynthesis. In terms of light energy capture, the top three leaves are the light energy capture center of cotton, while the fourth leaf is at a competitive disadvantage in the growth process. Nitrogen deficiency will intensify the competitive disadvantage of the top four leaves for light energy. However, with the increase of nitrogen nutrition, the top three leaves gradually approach the saturation state of growth, while the fourth leaf will continue to absorb nitrogen, thus narrowing the gap with the other top three leaves.

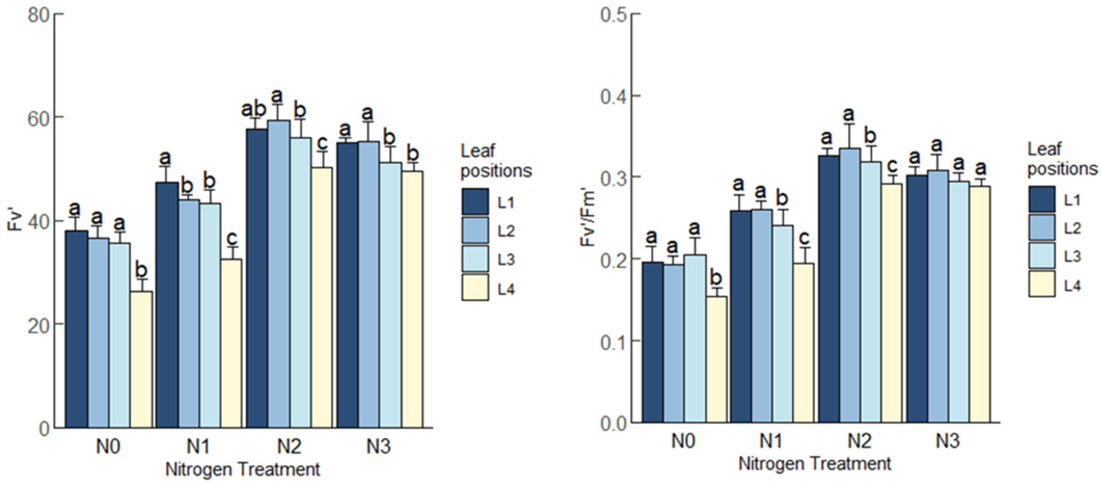

Figure 4. Changes in fluorescence parameters F0' and Fv'/Fm' of chlorophyll in different leaf positions of cotton at different nitrogen levels (flocculation stage)

Effect of nitrogen application level and leaf position on the photosynthetic parameters of leaves

The trends of net photosynthetic rate (Pn) and stomatal conductance (Gs) of cotton upper leaves with the N application level and the leaf position during the flocculation period were basically the same, and similar to the trends of the chlorophyll fluorescence parameters. Under different N application treatments, Pn in the top three leaves showed a significant increase in the N2 treatment, with increases of 13.71%, 18.33%, and 12.42% in the first, second, and third leaves, respectively, compared with the N0 treatment, while the difference between the N3 and N2 treatments was not significant. Compared to N0, Gs at different leaf positions also showed the most significant elevation under the N2 treatment. N1, from the first leaf to fourth leaf, increased by 10.86%, 17.71%, 16.24, and 19.27%, respectively, compared to N0. N2 increased by 30.33%, 34.93%, 31.64%, and 44.56%, respectively, and N3 increased by 16.91%, 31.35%, 34.02%, and 42.56%, respectively.

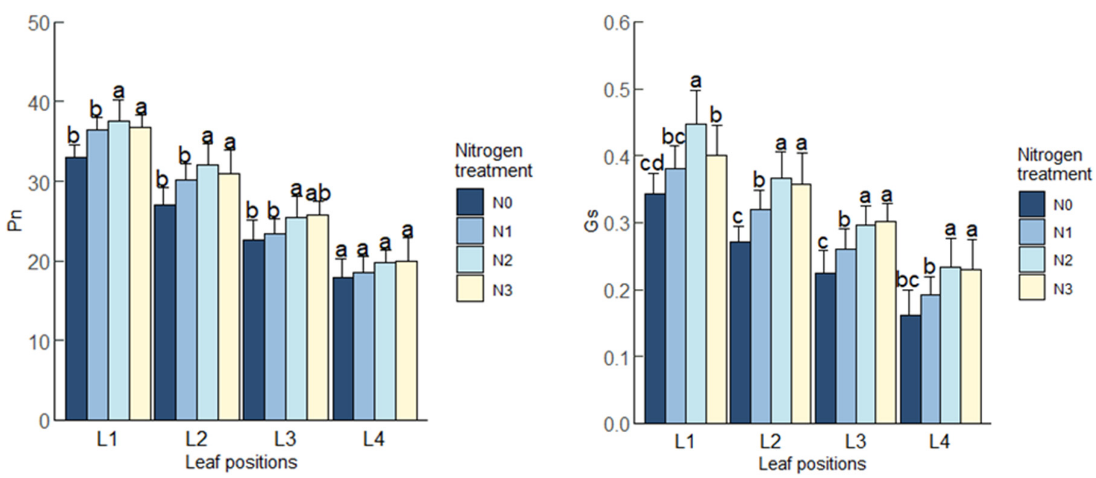

Figure 5. Changes in the net photosynthetic rate (Pn) and stomatal conductance (Gs) of cotton at different leaf positions under different nitrogen levels (flocculation stage)

Relationship between single leaf fluorescence parameters and yield composition

The initial fluorescence (F0) represents the size of the Photosystem II reaction center activity, and the maximum fluorescence (Fm) can reflect the electron transfer through PS II. Based on a comprehensive analysis of the correlation between the fluorescence parameters and the yield and yield components of the top four cotton leaves (Figure 6), the correlation coefficients of the top three leaves were significantly higher than those of the fourth leaf, but each yield component factor showed differences among different leaf positions, with the best correlation between boll number per plant and the Fv/F0 of the third leaf, with a correlation coefficient of 0.63. Regarding the single boll weight and the Fv/F0 of the second leaf, the correlation coefficient was 0.60, and the correlation coefficient between yield and Fv/F0 of the third leaf was the highest at 0.64. In general, the chlorophyll fluorescence parameters of cotton upper leaves were correlated with yield and yield composition factors, but the correlation coefficients were low and unstable among leaf positions.

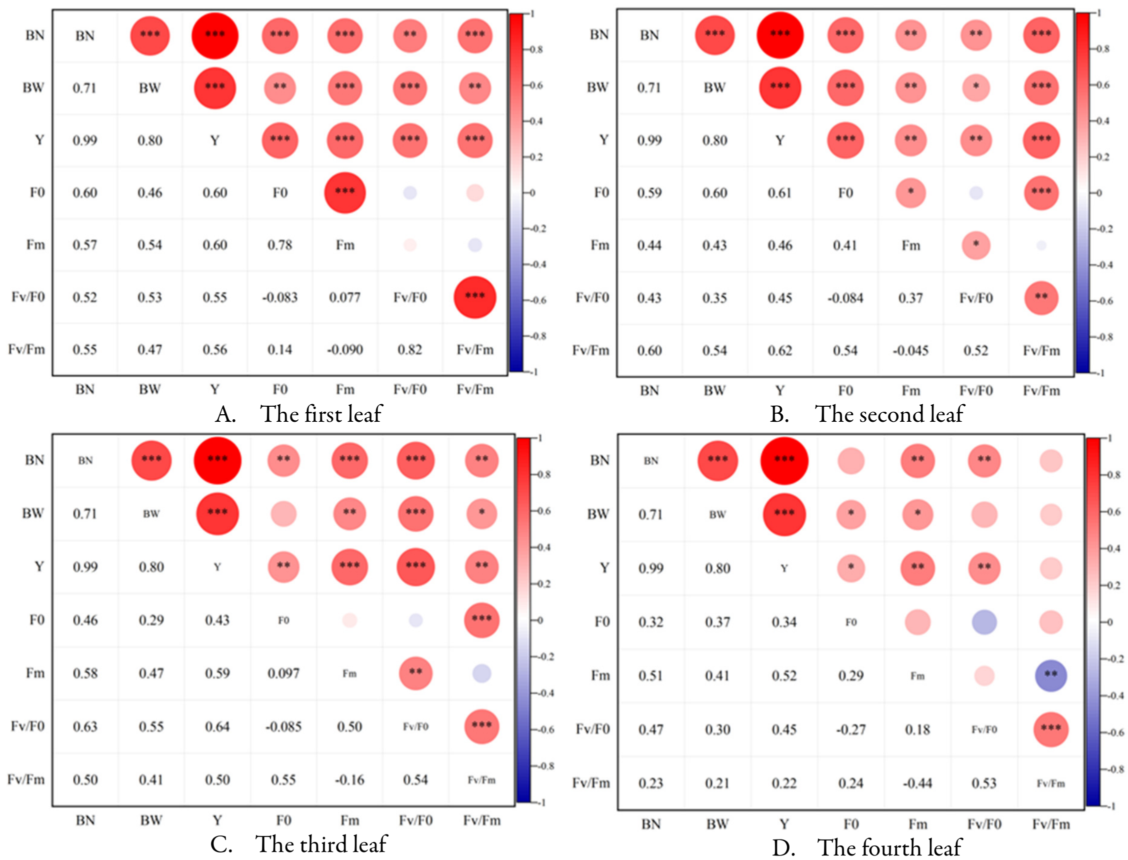

Figure 6. Correlation coefficients between the fluorescence parameters and the yield and yield components for the top four cotton leaves

Notes: ***, **, and * represent significant correlations at the probability level of $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively. BW represents boll weight, BN represents the number of bolls per plant, and Y represents yield.

Relationship between fluorescence accumulation and yield composition of single leaves

To obtain a better correlation with yield, each fluorescence parameter of the leaves was combined with the corresponding leaf area index to construct the leaf chlorophyll fluorescence accumulation. Compared with the fluorescence parameters, the correlation between fluorescence accumulation and yield was significantly higher, and the correlation was more stable (Figure 7). In terms of the correlation between fluorescence parameters of the first leaf and the yield and yield component factors, the correlation coefficients of the F0, Fm, Fv/F0, and Fv/Fm values of the first leaf with the number of individual bolls were 0.52-0.60, the correlation coefficients with the single boll weight were 0.46-0.54, and the correlation coefficients with yield were 0.55-0.60. The correlation coefficients of fluorescence accumulation corresponding to the first leaf with the number of individual bolls were 0.69-0.75, the correlation coefficients with the single boll weight were 0.50-0.65, and correlation coefficients with yield were 0.71-0.77. The fluorescence accumulation of each yield component factor increased by 15.00%-44.23%, 6.38%-22.64%, and 18.33%-40.00% compared to fluorescence parameters. The correlation coefficients between the fluorescence parameters and the yield of the second leaf were 0.45-0.62, corresponding to the correlation coefficients of 0.72-0.82 between the fluorescence accumulation and the yield, which increased by 29.51%-68.89% compared with the fluorescence parameters. The correlation coefficients between the fluorescence accumulation and the yield of the third leaf increased by 15.38%-131.82% compared with the fluorescence parameters, while the correlation coefficients of the fourth leaf did not increase. The correlation coefficients of the fourth leaf did not improve significantly and even decreased slightly. Overall, after combining the leaf area index, the correlation between the fluorescence accumulation and the yield of the top three leaves was significantly improved the compared with the fluorescence parameters, and the correlation was improved and more stable, while the fourth leaf showed no significant improvement, indicating that the top three leaves of cotton are the ideal leaf position for assessing yield when applying chlorophyll fluorescence signals.

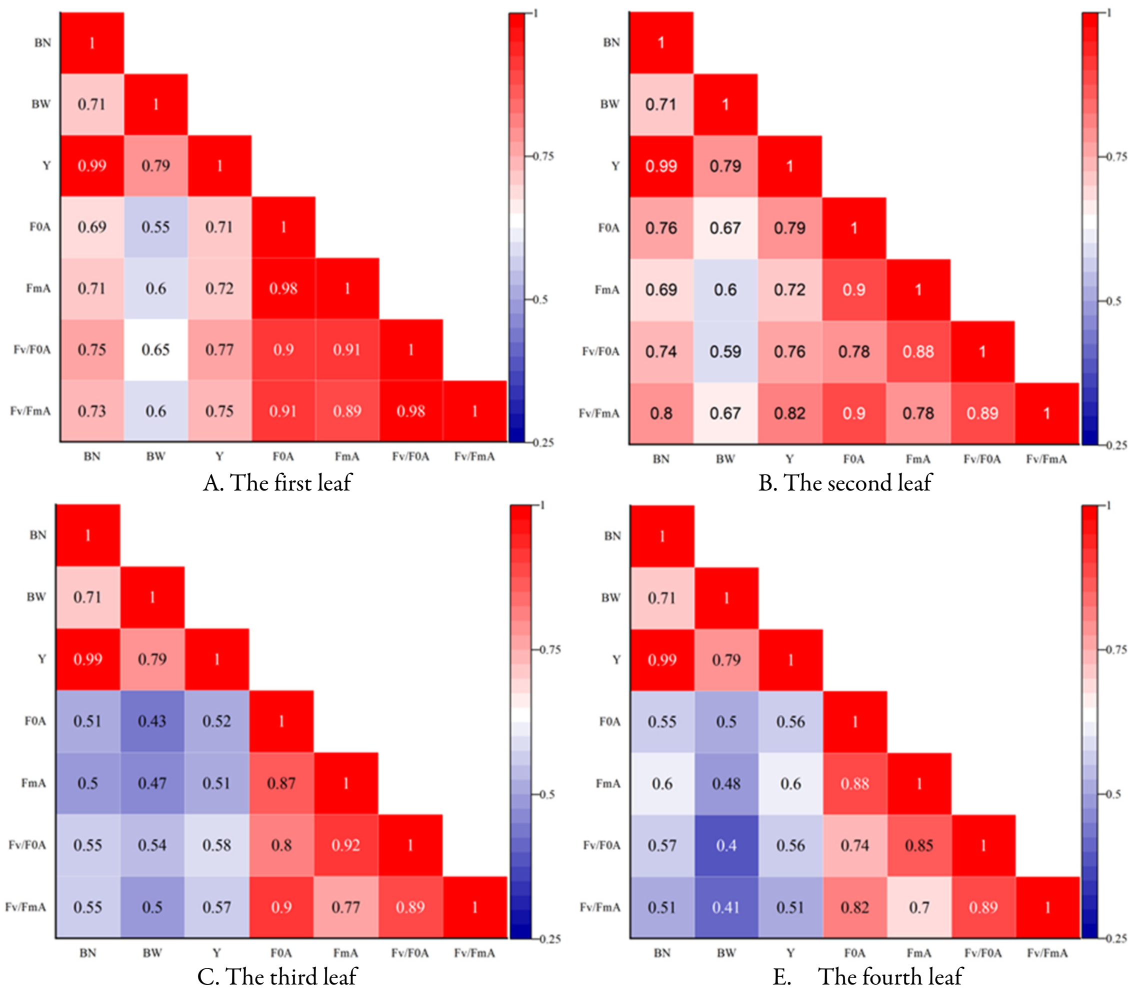

Figure 7. Correlation coefficients between the fluorescence parameters and the yield and yield components for the top four cotton leaves

Notes: ***, **, and * represent significant correlations at the probability level of P < 0.001, P < 0.01, and P < 0.05, respectively. BN represents the number of bolls per plant, BW represents boll weight, and Y represents Yield. F_0A represents F_0 accumulation. The accumulation of other fluorescence parameters was the same as the accumulation of F0.

Relationship between fluorescence accumulation of leaf combinations and yield composition

By combining the top four leaves in different ways (Table 2), it can be seen that for the combination of two adjacent leaves, the top two leaf combination (L12) showed the best correlation between fluorescence accumulation and yield. The correlation coefficients of F0 accumulation, Fm accumulation, Fv/F0 accumulation, and Fv/Fm accumulation with the number of bolls per plant ranged from 0.75 to 0.82. The correlation coefficients with boll weight ranged from 0.64 to 0.67. The correlation coefficients with yield were the highest, ranging from 0.77 to 0.84 for the top two leaves (L12), followed by L23 and L34, in that order. The correlation coefficients of Fm accumulation, Fv/F0 accumulation, and Fv/Fm accumulation with the number of individual bolls ranged from 0.75 to 0.82. The correlation coefficients with boll weight ranged from 0.64 to 0.67. The correlation coefficients with yield were the highest, ranging from 0.77 to 0.84, followed by L23 and L34, in that order. In summary, the correlations of the fluorescence accumulation with the yield and yield composition factors were higher for L12, L1-3, and L1-4, and the correlation coefficients were significantly higher than those of the single-leaf fluorescence accumulation, which can be used to better predict cotton yield.

Table 2. Characteristic wavelengths of different spectrum regions

Yield and yield composition factors	Leaf positions	F0 accumulation	Fm accumulation	Fv/F0 accumulation	Fv/Fm accumulation
Number of bolls per plant	L12	0.78**	0.75**	0.80**	0.82**
	L23	0.73**	0.68**	0.78**	0.80**
	L34	0.56**	0.57**	0.61**	0.58**
	L1-3	0.79**	0.75**	0.82**	0.83**
	L2-4	0.70**	0.68**	0.78**	0.78**
	L1-4	0.77**	0.76**	0.82**	0.82**
Boll weight	L12	0.66**	0.64**	0.67**	0.67**
	L23	0.64**	0.61**	0.68**	0.66**
	L34	0.49**	0.49**	0.51**	0.50**
	L1-3	0.67**	0.66**	0.71**	0.70**
	L2-4	0.62**	0.59**	0.64**	0.66**
	L1-4	0.66**	0.65**	0.69**	0.69**
Yield	L12	0.80**	0.77**	0.82**	0.84**
	L23	0.75**	0.70**	0.80**	0.81**
	L34	0.57**	0.58**	0.61**	0.59**
	L1-3	0.81**	0.78**	0.84**	0.85**
	L2-4	0.72**	0.70**	0.79**	0.79**
	L1-4	0.79**	0.78**	0.84**	0.83**

Construction of a model for cotton yield estimation based on the accumulation of chlorophyll fluorescence parameters

Based on the above study, leaf positions and leaf position combinations with correlation coefficients higher than 0.7 for each chlorophyll fluorescence accumulation and cotton yield were selected, namely L1, L2, L12, L1-3, and L1-4. Three machine learning algorithms were used to construct the estimation models of fluorescence accumulation and yield. The R2, RMSE, and n-RMSE of the model validation results are shown in Figure 8 and Table 3. The support vector machine and the BP neural network algorithm had a higher coefficient of determination and improved stability, and XGBoost performed relatively poorly. The BP algorithm had the highest coefficient of determination and the highest stability for the L12Fv/F0 cumulants with the yield estimation model, with R2=0.87, RMSE=64.84, and n-RMSE=8.54%, followed by the SVM algorithm for the L1-3F0 cumulants, with R2=0.84, RMSE=72.71, and n-RMSE=9.58%. Although the accuracy of the models constructed using the preferred fluorescence accumulation and yield were better under different algorithms, the model determination coefficients somewhat differed among different fluorescence parameters. For F0 accumulation, the SVM method had the highest accuracy, between 0.84 and 0.69 under different leaf positions, followed by BP and XGBoost, in that order; for Fm, Fv/F0, Fv/Fm accumulation, the BP method had the best performance, followed by SVM and XGBoost, in that order. The model accuracy of L2 was significantly higher than that of L1 in the estimation model of single-leaf fluorescence accumulation and yield. The model accuracy of the combined leaf fluorescence accumulation was significantly higher than that of single-leaf model. The model accuracy of L12 fluorescence accumulation under different modeling methods was the highest, followed by L1-3 and L1-4, in that order.

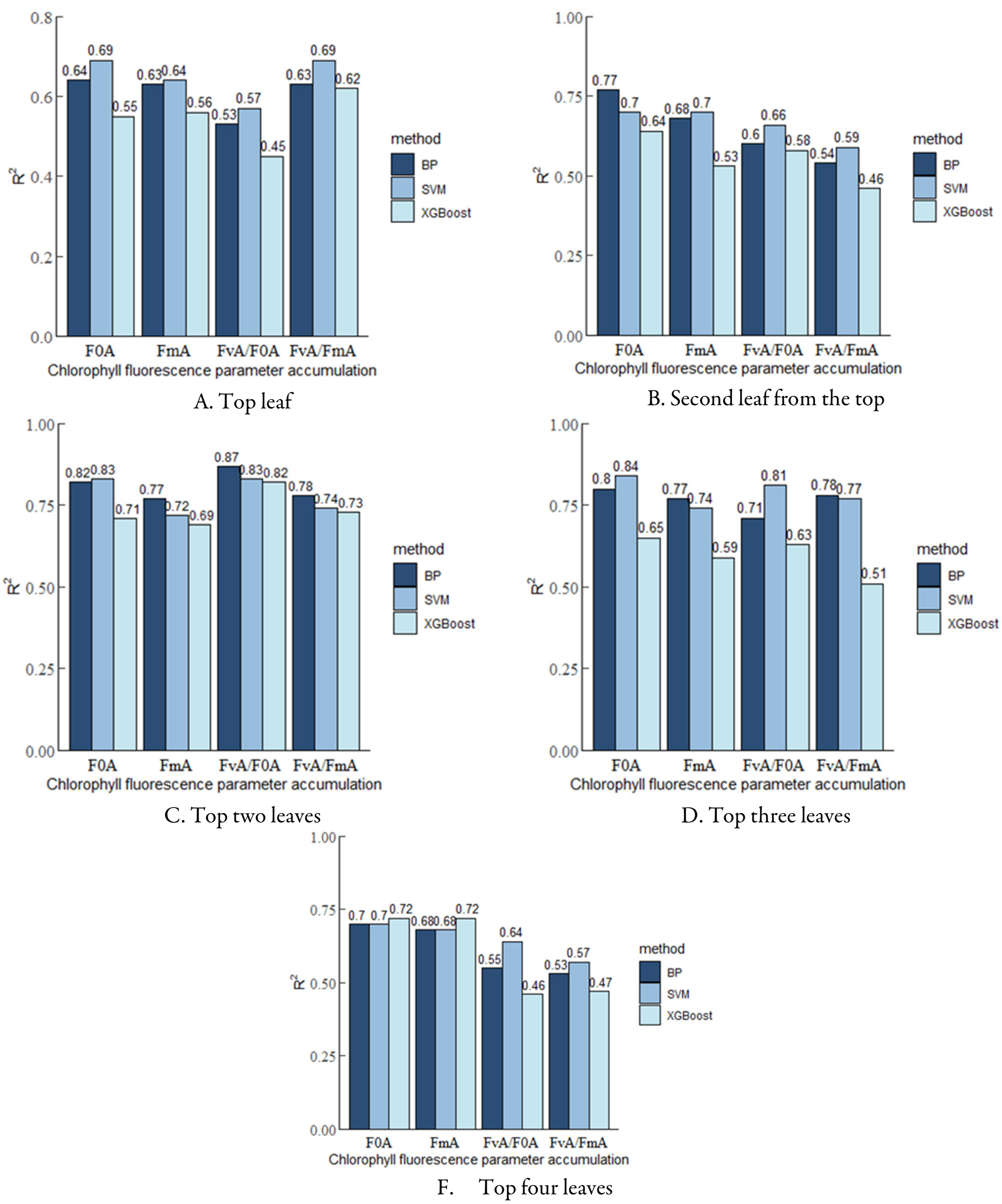

Figure 8 Evaluation of cotton yield estimation models based on different machine learning algorithms (R^2)

Table 3 shows that the RMSE and n-RMSE values of the prediction models for the leaves and leaf combinations and the yield, which were preferentially selected under different machine learning algorithms, were low. The n-RMSE values were below 20%, indicating that the deviation between simulated and measured values was small and that the models showed better regression performance. The L12Fv/F0 accumulation (BP), L12F0 accumulation (SVM), L1-3F0 accumulation (SVM), L12Fv/F0 accumulation (SVM), L12Fv/F0 accumulation (XGBoost), and yield estimation models had an n-RMSE of less than 10%, which indicates that the simulation performance of these models is excellent. Therefore, the models can be selected according to different fluorescence parameters on merit. Overall, the models constructed with the SVM and the BP neural network have higher coefficients of determination and are suitable for the chlorophyll fluorescence estimation of cotton yield, but the model constructed with the SVM has better stability.

Table 3. Evaluation of cotton yield estimation models based on different machine learning algorithms (RMSE and n-RMSE)

Method	Chlorophyll fluorescence	RMSE					n-RMSE				
		L1	L2	L12	L1-3	L1-4	L1	L2	L12	L1-3	L1-4
BP	F0 accumulation	106.02	85.82	76.03	78.55	96.40	13.97%	11.30%	10.01%	10.35%	12.70%
	Fm accumulation	108.27	100.28	84.93	85.76	106.47	14.26%	13.21%	11.19%	11.30%	14.02%
	Fv/F0 accumulation	121.48	112.70	64.86	94.92	118.36	16.00%	14.85%	8.54%	12.50%	15.59%
	Fv/Fm accumulation	107.34	119.60	83.03	83.79	121.65	14.14%	15.75%	10.94%	11.04%	16.02%
SVM	F0 accumulation	98.16	97.68	74.17	72.71	96.84	12.93%	12.87%	9.77%	9.58%	12.76%
	Fm accumulation	105.80	108.50	92.97	90.06	100.71	13.94%	14.29%	12.25%	11.86%	13.27%
	Fv/F0 accumulation	115.89	104.41	73.29	77.57	106.45	15.27%	13.75%	9.65%	10.22%	14.02%
	Fv/Fm accumulation	97.90	113.30	90.23	85.50	115.89	12.90%	14.92%	11.89%	11.26%	15.27%
XGBoost	F0 accumulation	118.58	105.84	94.89	105.21	93.49	15.62%	13.94%	12.50%	13.86%	12.31%
	Fm accumulation	116.98	121.66	99.08	113.28	93.79	15.41%	16.03%	13.05%	14.92%	12.36%
	Fv/F0 accumulation	131.40	115.16	74.31	107.35	129.75	17.31%	15.17%	9.79%	14.14%	17.09%
	Fv/Fm accumulation	109.34	144.47	92.28	123.69	129.32	14.40%	19.03%	12.16%	16.29%	17.04%

Discussion

Under environmental or nutrient stress conditions, the photosynthetic system is disrupted, Photosystem II activity is affected, and leaf photosynthetic and chlorophyll fluorescence parameters are altered in crops. Sun et al. (Sun *et al*., 2020) found that the Fv/Fm and photosynthetic performance index of leaves at the staminode stage were sensitive to nitrogen status, and multicolor fluorescence imaging had the best accuracy in the early diagnosis of nitrogen status. Hutmacher *et al*. (2001) found that the cotton yields tended to increase when between 50 and 224 kg-hm^{-2} of N was applied, while excessive N application decreased yields. In the present study, the fluorescence parameters Fv' and Fv'/Fm' increased continuously with the increase of N application in the range of 0–240 kg-hm^{-2} during the cotton spatulation period, and started to decrease when the N application exceeded 240 kg-hm^{-2}. The trends of the photosynthetic parameters Pn and Gs with the nitrogen application level and the leaf position were basically the same, and they were similar to those of chlorophyll fluorescence parameters. Both Pn and Gs decreased as leaf position decreased, and they tended to increase and then slowly decrease as nitrogen application increased. Taken together, a moderate N application improved the Photosystem II activity of the plants.

Leaf photosynthesis is the basis of crop yield formation. Chlorophyll fluorescence technology can reliably monitor the photosynthetic capacity of crops (Murchie *et al.*, 2013) and has been widely used in crop growth and development as well as in adversity stress (Wang *et al.*, 2019; Dong *et al.*, 2020; Baha, 2021). Živčák *et al.* (2014) evaluated the effect of nitrogen deficiency on the photosynthetic performance of wheat using chlorophyll fluorescence parameters and showed that the maximum photochemical quantum yield of wheat leaves was less sensitive to nitrogen application. In this study, the correlation between fluorescence parameters and cotton yield and constitutive factors was analyzed. It was found that the fluorescence parameters F0, Fm, Fv/F0, and Fv/Fm were significantly and positively correlated with yield and constitutive factors in the first and the second leaves of cotton at the spatulation stage. Therefore, the fluorescence parameters of the upper leaves were combined with the leaf area index to construct the leaf fluorescence accumulation, which was used to further evaluate the cotton yield status. The correlation coefficient between fluorescence accumulation and yield was significantly higher compared with that of fluorescence parameters, indicating that adding the growth information of the leaf area index to the physiological index of photosynthetic fluorescence parameters and making full use of the advantages of both is important in estimating cotton yield.

There is a clear spatial heterogeneity in the plant canopy, and different optical and photochemical properties of leaves lead to differences in physiology, growth parameters, and photosynthetic capacity in different leaf positions (Bussotti *et al.*, 2011). The basis and manner of selecting leaf positions in previous studies vary depending on the objectives and content of the study. This study showed that fluorescence parameters F0, Fm, Fv/F0, Fv/Fm, Pn, and Gs differed significantly among different leaf positions. They generally decreased as leaf position decreased, and the fluorescence parameters of the first and the second leaves were higher and significantly higher than those of the third and the fourth leaves, respectively, which was mainly due to the spatial structure of the upper leaves of cotton, as the leaves in the upper part receive more light. This is mainly due to the spatial structure of the upper leaves of cotton and the increased amount of light that the upper leaves receive. The senescence of the leaves starts from the lower leaves. Although studies have shown that single leaves can provide information on crop growth, the stability of the results needs to be further improved. Therefore, in order to improve the accuracy and universality of crop growth information inversion, optimizations involving the combination of leaf positions have received increasing attention. Tian *et al.* (2005) found that the top two leaves of rice were an optimal combination for evaluating the photosynthetic capacity of the plant. In this study, the fluorescence accumulation of different leaf positions was combined to obtain the combined fluorescence accumulation of leaves to further improve the accuracy of yield estimation. The results showed that, for single leaves, the correlation between fluorescence accumulation and yield decreased as leaf position decreased; for leaf combinations, the correlation coefficients between the fluorescence parameter accumulation and the yield were ordered as follows: L12 > L1-3 > L1-4 > L23 > L2-4 > L34. This indicated that the combination of the first and second leaves is more responsive to yield and can be used to reliably evaluate cotton yield. The fourth leaf may carry redundant and messy information and cannot estimate yield as reliably. In this study, the correlation between the accumulation of four chlorophyll fluorescence parameters (F0, Fm, Fv/F0, and Fv/Fm) and yield was the most significant, followed by the accumulation of Fv/Fm. The above parameters can be used to construct a model for estimating the yield of cotton.

Previous studies have verified that chlorophyll fluorescence techniques can identify different water and nitrogen states in plants, and the classification of nine different water and nitrogen coupling states was accomplished using support vector machine (SVM), radial basis function (RBF), and neural network (BP) methods (Zhou *et al.*, 2018). In this study, leaves and leaf combinations (L1, L2, L1-2, L1-3, and L1-4) with a good correlation with yield were selected, and three machine learning algorithms were used to establish estimation models of the chlorophyll fluorescence parameters of the upper leaves and cotton yield. The results showed that the SVM and BP models were superior to the XGBoost regression tree. The BP algorithm had the highest coefficient of determination and the highest stability for the model of L12Fv/F0 accumulation and yield estimation, with R^2=0.87, RMSE=64.84, and n-RMSE=8.54%. The correlation coefficients of L12Fv/F0 accumulation, L12Fv/Fm accumulation, L1-3Fv/F0 accumulation, and L1-3Fv/Fm accumulation with yield were 0.82, 0.845, 0.84, and 0.85, respectively, which shows that the correlation between the L1-3 combination and yield was superior to that of the L12 combination and yield, and the correlation between Fv/Fm accumulation and yield was superior to that of Fv/F0 accumulation and yield. However, the R^2 values of the model under different methods in the yield estimation model were 0.82~0.87, 0.73~0.78, 0.63~0.81, and 0.51~0.78, indicating that the yield estimation performance of L12 was more stable compared with L1-3, and the stability of the Fv/F0 cumulative yield estimation was also superior to that of the Fv/Fm cumulative yield estimation. Overall, the fluorescence accumulation and yield estimation model based on the combination of the first and the second leaves had the highest accuracy and stability and could better evaluate the cotton yield status.

Chlorophyll fluorescence technology can capture the subtle changes of crop physiology in a timely and accurate manner and is known to be a probe of photosynthesis. In this study, we took advantage of the active nature of chlorophyll fluorescence, combined it with the spatial characteristics of the cotton canopy structure and an analysis of differences between upper leaf positions, and realized the predictive evaluation of cotton yield by studying the accumulation of fluorescence in the upper leaves of cotton. However, the determination of chlorophyll fluorescence parameters can be affected by the measurement environment, and the data used in this study involved a single test site, a single variety, and four nitrogen gradients in a single year. Future studies need to combine different sites, different varieties, and more nitrogen levels in their experiments, and the model needs to be further improved to enhance reliability and applicability.

Conclusions

This study formed fluorescence accumulation data based on chlorophyll fluorescence parameters and the leaf area index of the top four leaves of cotton, and the cotton yield was estimated by using three machine learning methods: a support vector machine, an artificial neural network, and XGBoost. The results showed that there was a significant positive correlation between the fluorescence parameter Fv/Fm accumulation and the cotton yield in the top three leaves combined, with a correlation coefficient of 0.849. The results of cotton yield estimation models with different modeling methods showed that the SVM and BP neural network obtained improved regression results, among which the L12Fv/F0 accumulation in the BP neural network algorithm The estimation model with yield had the highest coefficient of determination and the highest stability, and the model evaluation index results were R^2=0.87, RMSE=64.84, and n-RMSE=8.54%, which are more suitable for datasets with fluorescence accumulation parameters related to cotton leaves.

Authors' Contributions

YRD were responsible for determination of some indexes and the writing of this manuscript. SZQ, LLM and XYC performed the experiments and collected all data sets. QSY, MY and YRM were responsible for the mapping and typesetting. XL were responsible for the revision. ZZ, the corresponding author, were responsible for the revision and quality control of the paper. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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