

## Analysis of Vertical Heterogeneity of Biochemical Parameters in Rapeseed Canopy Based on Rapid Chlorophyll Fluorescence Technology (Postprint)

**Authors:** Jiafei Zhang, Wan Liang, He Yong, Cen Haiyan

**Date:** 2023-02-17T00:00:00+00:00

### Abstract

Accurate acquisition of biochemical information from crop canopies is of great significance for monitoring crop growth and guiding precision fertilization. Existing research on the vertical distribution of crop biochemical parameters primarily focuses on hyperspectral remote sensing inversion, lacking connection with photosynthetic physiology. This study mainly investigated the vertical distribution characteristics of biochemical parameters such as chlorophyll, carotenoids, dry matter, and water content within oilseed rape canopies at the seedling stage under different nitrogen treatment levels, while simultaneously using rapid chlorophyll fluorescence technology to measure leaf photosynthetic performance, and further analyzing the intrinsic relationship between fluorescence response and biochemical parameters through linear regression analysis and principal component analysis. The experimental results showed that: (1) Chlorophyll content, carotenoid content, dry matter, and water content in oilseed rape canopies at the mid-seedling stage all exhibited parabolic non-uniform vertical distributions, whereas the chlorophyll to carotenoid ratio displayed a distinct vertical distribution pattern from other biochemical parameters, gradually decreasing with increasing leaf position and nitrogen application rate, which was consistent with the vertical distribution patterns of fluorescence parameters such as driving force  $DF_{Total}$  and quantum yield at the end of electron transport chain  $\phi_{Ro}$ ; (2) Fluorescence parameters, particularly  $DF_{Total}$ , demonstrated strong evaluation capability for the chlorophyll to carotenoid ratio, chlorophyll content, and dry matter content in oilseed rape leaves; (3) Nitrogen deficiency reduced the performance of photosystem I and II (PSI and PSII) in oilseed rape leaves at the seedling stage, and nitrogen stress could be diagnosed through fluorescence parameters such as maximum photochemical efficiency  $\phi_{Po}$ ; moreover, leaves at different positions showed significant differences in PSI performance, i.e., electron transport efficiency at the terminal end, and  $DF_{Total}$  could effectively characterize the vertical heterogeneity of canopy biochemical parameters.

These results demonstrate that applying rapid chlorophyll fluorescence technology for vertical heterogeneity monitoring of biochemical information in crops is feasible, and can provide new ideas and technical support for guiding precision fertilization and improving high quality and high yield.

## Full Text

### Vertical Heterogeneity Analysis of Biochemical Parameters in Oilseed Rape Canopy Based on Fast Chlorophyll Fluorescence Technology

ZHANG Jiafei<sup>1,2</sup>, WAN Liang<sup>1,2</sup>, HE Yong<sup>1,2,3</sup>, CEN Haiyan<sup>1,2,3\*</sup>

<sup>1</sup> College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China

<sup>2</sup> Key Laboratory of Spectroscopy Sensing, Ministry of Agriculture and Rural Affairs, Hangzhou 310058, China

<sup>3</sup> State Key Laboratory of Modern Spectroscopic Instruments, Zhejiang University, Hangzhou 310027, China

---

## Abstract

Accurate acquisition of crop canopy biochemical information is of great significance for monitoring crop growth and guiding precision fertilization. Existing research on the vertical distribution of crop biochemical parameters has primarily focused on hyperspectral remote sensing inversion, lacking connection with photosynthetic physiology. This study investigated the vertical distribution characteristics of biochemical parameters—including chlorophyll, carotenoids, dry matter, and water content—in oilseed rape canopies under different nitrogen treatments during the mid-seedling stage. Concurrently, leaf photosynthetic performance was measured using fast chlorophyll fluorescence technology, and linear regression analysis and principal component analysis were employed to further explore the intrinsic relationships between fluorescence responses and biochemical parameters. The experimental results demonstrated that: (1) Chlorophyll content, carotenoid content, dry matter, and water content in the rape canopy at the mid-seedling stage all exhibited non-uniform parabolic vertical distributions, while the chlorophyll-to-carotenoid ratio showed a distinct vertical distribution pattern, gradually decreasing with increasing leaf position and nitrogen application rate—matching the vertical distribution patterns of fluorescence parameters such as driving force  $DF_{Total}$  and end-chain quantum yield  $\varphi_{Ro}$ ; (2) Fluorescence parameters, particularly  $DF_{Total}$ , exhibited strong evaluation capabilities for the chlorophyll-to-carotenoid ratio, chlorophyll content, and dry matter content in rape leaves; (3) Nitrogen deficiency reduced the Photosystem II (PSII) and Photosystem I (PSI) performance of rape leaves at the seedling stage, and fluorescence parameters such as maximum photochemical efficiency  $\varphi_{Po}$  could

diagnose nitrogen stress, while leaves at different positions showed significant differences in PSI performance (i.e., electron transfer efficiency at the acceptor side), which could be effectively characterized by  $DF_{Total}$  to represent vertical heterogeneity of canopy biochemical parameters. These findings indicate the feasibility of applying fast chlorophyll fluorescence technology for vertical heterogeneity monitoring of crop biochemical information, providing new insights and technical support for guiding precision fertilization and achieving high-quality, high-yield production.

**Keywords:** fast chlorophyll fluorescence transient; JIP-test; vertical heterogeneity; chlorophyll; carotenoid; nitrogen

---

## 1 Introduction

Oilseed rape is the world's second-largest oil crop after soybean, playing a crucial role in global petroleum resource substitution, animal feed, and biofuel supply [1]. Rapid acquisition of biochemical phenotypic information (e.g., pigments, dry matter, water content) is essential for breeding superior varieties and improving crop quality and yield. Leaf biochemical characteristics often exhibit significant non-uniform vertical distribution within canopies; for instance, nitrogen concentration is typically higher in upper leaves with more active photosynthesis [2,3]. This spatial heterogeneity within crop canopies can be viewed as an adaptive strategy that continuously adjusts allocation among leaves under limited nutrient resources and changing environmental conditions to maximize canopy photosynthetic rates [2,4]. Therefore, quantitative research and analysis of vertical heterogeneity of biochemical components within crop canopies are necessary to provide effective theoretical methods and technical support for dynamic monitoring of rape growth information and precision scientific fertilization.

Current research on vertical distribution of crop biochemical components primarily focuses on hyperspectral remote sensing for assessing nitrogen vertical distribution, establishing relationships between canopy spectra and nitrogen content in various vertical layers through statistical models [5-8]. However, reflectance-based monitoring methods often contain mixed signals from plants and soil, reducing inversion accuracy and robustness, and the connection with physiological mechanisms such as photosynthesis is not well understood [9,10]. Moreover, research on vertical distribution of biochemical components closely related to photosynthetic physiology, such as photosynthetic pigments, is relatively scarce, failing to meet the requirements for high-throughput phenotypic information acquisition in field crop breeding and nitrogen management during critical growth stages.

In plant photosynthesis, energy absorbed by chlorophyll molecules is generally converted through three pathways: photochemistry, thermal dissipation, and chlorophyll fluorescence [11]. In other words, the remaining light energy not

consumed by photochemical reactions or converted to heat is radiated from the plant as fluorescence. Plant senescence or stress conditions—including high/low temperature, salinity, and drought—can cause changes in these three energy forms [12,13]. Therefore, changes in chlorophyll fluorescence can detect variations in leaf physiological status, such as light energy absorption and transfer, reaction center status, excess energy and its dissipation, PSII donor and acceptor side activity, and photosynthetic photoinhibition and photodamage [14,15].

Compared with traditional gas exchange measurement methods, chlorophyll fluorescence technology has become a more effective, convenient, and high-throughput approach for understanding spatiotemporal dynamics of photosynthetic function and physiological characteristics. Its main advantage lies in that fluorescence signals originate only from plants themselves, effectively avoiding interference from other signals such as soil and directly reflecting the most authentic plant physiological status. Beyond detecting plant stress responses, numerous studies have applied chlorophyll fluorescence to analyze physiological and ecological indicators such as crop chlorophyll content and nitrogen nutrient index (NNI). Fluorescence parameters are significantly affected by nutrient supply such as nitrogen; for example, non-photochemical quenching (NPQ) increases with nitrogen application in rice [16], while excessive nitrogen application in cotton causes declines in maximum photochemical efficiency ( $F_v/F_m$ ) and electron transport rate (ETR) [17]. Additionally, some studies have observed significant differences in PSII activity and energy utilization efficiency across different leaf layers using fluorescence parameters [18,19], demonstrating the sensitivity and great potential of chlorophyll fluorescence response for characterizing crop photosynthetic physiology, particularly vertical heterogeneity. However, research on vertical heterogeneity of fluorescence parameters within crop canopies remains limited. Therefore, exploring the vertical heterogeneity of fluorescence parameters within crop canopies and their relationship with biochemical information using chlorophyll fluorescence technology holds considerable promise as an alternative method for evaluating crop biochemical parameters.

This study primarily used spectrophotometry to measure and analyze the vertical distribution characteristics of biochemical parameters such as chlorophyll, carotenoids, dry matter, and water content in oilseed rape canopies during the seedling stage. Concurrently, fast chlorophyll fluorescence technology was employed to investigate vertical heterogeneity of photosynthetic physiological characteristics within the canopy, analyze the intrinsic connections and physiological mechanisms between fluorescence responses and biochemical parameters, and ultimately identify the most suitable fluorescence parameters for characterizing rape biochemical information.

## 2 Materials and Methods

### 2.1 Experimental Design

The experimental material was the *Brassica napus* L. cultivar Zheshuang 758. The field experiment was conducted at the Agricultural Experimental Station of Zhejiang University in Zijingang Campus. Three nitrogen levels were established: no nitrogen (N0, 0 kg N/ha), normal nitrogen (N1, 215 kg N/ha), and excessive nitrogen (N2, 430 kg N/ha), with a ratio of N0:N1:N2 = 0:1:2. Nitrogen fertilizer was applied at a 3:1:2 ratio before transplanting, at the early seedling stage, and at the bolting stage, respectively. Phosphorus and potassium fertilizers were applied as basal fertilizers. The experimental field used a randomized block design with three replicates for each nitrogen level, totaling nine plots. Each plot area was 6.6 m<sup>2</sup> (5.5 m × 1.2 m), with 0.4 m wide protective rows in both north-south and east-west directions. Oilseed rape was transplanted in early November 2019, and the experiment was conducted on January 6, 2020 (mid-seedling stage).

### 2.2 Measurement Methods

**2.2.1 Fast Chlorophyll Fluorescence Measurement** In each of the three plots for each nitrogen level, three rape samples with similar and healthy growth were randomly selected. Leaves along the main stem were marked from bottom to top as L1 (bottom 1st leaf), L2 (bottom 2nd leaf), L3 (bottom 3rd leaf), L4 (bottom 4th leaf), L5 (bottom 5th leaf), L6 (bottom 6th leaf), and L7 (bottom 7th leaf). Based on their relative vertical positions, they were classified as basal leaves (L1, L2), middle leaves (L3-L5), and top leaves (L6, L7). During measurement, a point in the upper-middle region on the right side of the leaf vein was selected as the sampling point. In the field, a leaf clip (diameter 4 mm) was used for 20 minutes of dark adaptation at the sampling point, followed by measurement of fast chlorophyll fluorescence induction kinetics using a Handy-PEA (Handy Plant Efficiency Analyzer, Hansatech Instruments Ltd., UK).

**2.2.2 Biochemical Parameter Measurement** After completing field measurements, leaf samples from each leaf position were collected. In the laboratory, sampling points from each leaf position were punched with a 0.85 cm diameter hole punch and weighed for fresh weight. Pigments were extracted by soaking in 1.8 mL of 95% ethanol in darkness for 24 hours. Chlorophyll a, chlorophyll b, and carotenoid contents were measured using a spectrophotometer (Epoch, BioTek Instruments, Winooski, USA). Samples that completed pigment measurement were then dried in a 60°C oven to constant weight and weighed for dry matter. Water content was calculated by subtracting dry weight from fresh weight.

## 2.3 Data Analysis

**2.3.1 OJIP Transient and JIP-test Parameters** When green plants or chlorophyll-containing tissues are dark-adapted and suddenly exposed to visible light, they emit a dark-red fluorescence signal with changing intensity, known as the Kautsky effect [20]. During this process, fluorescence intensity first rises and then declines. The minimum fluorescence immediately after light exposure is defined as point O, the peak as point P, with two intermediate steps at point J (2 ms) and point I (30 ms). The fast chlorophyll fluorescence induction kinetics (OJIP) curve refers to the fluorescence change from point O to point P [21,22].

Changes in OJIP curve morphology reflect variations in the electron transport chain of photosynthetic primary reactions and the structure and state of photosynthetic apparatus in plant leaves. The JIP-test method enables quantitative analysis and physiological interpretation of these changes. Its theoretical foundation is the biomembrane energy flow model: antenna pigments convert a small portion of absorbed energy (ABS) into heat and fluorescence, while the remainder is trapped (TR) by reaction centers (RC) to reduce the oxidized primary electron acceptor  $Q_A$  to  $Q_A^-$ , which continues downward through electron transport (ET) to form an electron transport chain [23]. A series of chlorophyll fluorescence parameters are derived from this process. The specific calculation formulas and parameter definitions are shown in Table 1 [21].

**2.3.2 Principal Component Analysis** Principal Component Analysis (PCA) processes multivariate data by utilizing a “dimensionality reduction” approach to transform multiple indicators into a few comprehensive indicators [24]. The JIP-test based on OJIP curves involves numerous parameters, some of which are highly correlated, increasing analytical complexity. Therefore, PCA was employed to transform data from high-dimensional space to low-dimensional space, facilitating further statistical analysis of fluorescence parameters across different nitrogen treatments and leaf positions.

**2.3.3 Data Processing and Statistical Analysis** Significant differences in leaf biochemical parameters under different nitrogen treatments and leaf positions were tested using the Least Significant Difference (LSD) method ( $P < 0.05$ ). PCA of JIP-test parameters, correlation analysis between biochemical and fluorescence parameters, and significance analysis were all performed using SPSS Statistics 19 software (IBM Corporation, USA).

### 3 Results and Analysis

#### 3.1 Vertical Heterogeneity of Biochemical Parameters in Oilseed Rape Canopy

The variations in leaf chlorophyll content ( $C_{ab}$ ), carotenoid content ( $C_{xc}$ ), chlorophyll-to-carotenoid ratio ( $C_{ab}/C_{xc}$ ), chlorophyll a/b ratio (Chl a/b), dry matter content ( $C_m$ ), and water content ( $C_w$ ) at different leaf positions during the mid-seedling stage are shown in Figure 1 [Figure 1: see original paper]. These biochemical parameters all changed significantly across leaf positions, demonstrating vertical heterogeneity, with consistent vertical spatial patterns across different nitrogen treatments.

Specifically, chlorophyll (Figure 1(a)) and carotenoid (Figure 1(b)) contents exhibited downward-opening parabolic patterns from bottom to top, while dry matter (Figure 1(e)) and water content (Figure 1(f)) showed upward-opening parabolic patterns. This indicates that middle leaves (L3-L5) had the highest chlorophyll and carotenoid contents, while basal leaves (L1, L2) had higher chlorophyll and water contents but lower carotenoid and dry matter contents compared to top leaves (L6, L7). Since basal leaves were partially shaded and received less light intensity than top leaves, they required relatively less carotenoids for photoprotection. Notably, the normal nitrogen group (N1) had higher chlorophyll and carotenoid contents in basal leaves than both the nitrogen-deficient (N0) and excessive nitrogen (N2) groups, while middle leaves had the lowest values for chlorophyll, carotenoids, dry matter, and water content. Additionally, top leaf chlorophyll content tended to decrease with increasing nitrogen application. These results indicate that while nitrogen stress did not significantly affect the vertical distribution patterns of biochemical parameters in the rape canopy, under nitrogen deficiency, rape prioritized nitrogen allocation to top leaves to meet growth demands [25], whereas under sufficient nitrogen, more resources were supplied for continuous growth of new top leaves, resulting in higher chlorophyll content in top leaves of the nitrogen-deficient group.

The chlorophyll-to-carotenoid ratio (Figure 1(c)) showed a significant decreasing trend from bottom to top, reflecting higher carotenoid content and lower chlorophyll content in top leaves, with the opposite pattern in basal leaves. This ratio effectively reflects the photoprotective xanthophyll cycle response [26,27], indicating more active xanthophyll cycling for photoprotection in top leaves receiving more light energy. Furthermore, the ratio gradually decreased with increasing nitrogen application, suggesting a potential linear relationship with both leaf position (leaf age) and nitrogen level. The chlorophyll a/b ratio (Figure 1(d)) reflects antenna size, which plants adjust to adapt to different light intensities or nitrogen supply conditions. In basal leaves, the chlorophyll a/b ratio was significantly lower than in middle and top leaves, and generally increased with nitrogen application across leaf positions, reflecting larger antenna sizes (greater proportion of light-harvesting chlorophyll b) in basal leaves to

adapt to lower light intensities [28].

### 3.2 Vertical Heterogeneity of Photosynthetic Physiology Based on JIP-test Parameters

Using the JIP-test parameter values of the bottom leaf (L1) as a baseline (assigned as 1), the parameter values of other leaf positions were divided by this baseline to observe differences in JIP-test parameters across leaf positions, with results for different nitrogen treatments shown in Figure 2 [Figure 2: see original paper]. The radar plot revealed that important JIP-test parameters such as  $DF_{Total}$ ,  $\varphi_{Ro}$ ,  $RE_0/RC$ ,  $S_m$ , and  $N$  showed minimum values in top leaves, indicating weakest end-chain electron transport performance in top leaves. In nitrogen-deficient (N0) and normal nitrogen (N1) groups, heat dissipation per reaction center or per cross-section ( $DI_0/RC$  and  $DI_0/CS_m$ ) was highest in top leaves, as light energy absorbed by leaves increased with leaf position, and top leaves absorbed more light energy resulting in higher thermal dissipation. However, this value was not highest in the excessive nitrogen group (N2), possibly due to higher chlorophyll a/b ratios in top and middle leaves of N2, indicating relatively smaller antenna sizes (less light-harvesting chlorophyll b) that did not capture more light energy than other leaf positions.

### 3.3 Correlation Analysis Between Biochemical Parameters and JIP-test Parameters

To further investigate potential relationships between rape leaf biochemical parameters and JIP-test parameters, Pearson correlation analysis was performed, with results shown in Figure 3 [Figure 3: see original paper]. The logarithm of the chlorophyll-to-carotenoid ratio showed stronger correlations with JIP-test parameters than the ratio itself. Chlorophyll content, chlorophyll-to-carotenoid ratio,  $\log(C_{ab}/C_{xc})$ , and dry matter content showed high correlations with multiple JIP-parameters, while carotenoid content, chlorophyll a/b ratio, and water content showed weaker correlations.

Specifically,  $\delta_{Ro}$ ,  $\varphi_{Ro}$ , and  $DF_{Total}$  showed highly significant positive correlations with chlorophyll content ( $r > 0.5$ ,  $P < 0.01$ ).  $DF_{Total}$ ,  $\varphi_{Ro}$ ,  $PI_{ABS}$ ,  $S_m$ , and  $RE_0/RC$  all showed highly significant positive correlations with both the chlorophyll-to-carotenoid ratio and its logarithm.  $ABS/RC$  showed a highly significant positive correlation with dry matter content, while  $\delta_{Ro}$  and  $DF_{Total}$  showed highly significant negative correlations ( $r < -0.5$ ,  $P < 0.01$ ). These results indicate that increasing chlorophyll content and its ratio to carotenoids significantly enhances quantum yield and driving force for electron transfer to the PSI end-chain in primary photochemical reactions, while excessive dry matter and insufficient water content hinder electron transport, as reduced water content (the initial electron donor) affects the water photolysis process in photosynthesis.

Linear regression results for three JIP-test parameters highly correlated with

$\log(C_{ab}/C_{xc})$  (Figure 4(a)-(c)), chlorophyll content (Figure 4(d)-(f)), and dry matter content (Figure 4(g)-(i)) revealed that all three biochemical parameters showed significant linear relationships with  $DF_{Total}$  ( $P < 0.0001$ ), with coefficients of determination ( $R^2$ ) of 0.45, 0.35, and 0.27, respectively. Based on scatter distribution across leaf positions, the logarithm of the chlorophyll-to-carotenoid ratio effectively distinguished basal from top leaves compared to chlorophyll and dry matter content, further demonstrating the potential of  $DF_{Total}$  to reflect vertical heterogeneity in photoprotective xanthophyll cycling and other photosynthetic physiological processes.

### 3.4 PCA-based Clustering Analysis of JIP-test Parameters

Principal component analysis of JIP-test parameters from rape leaves at the mid-seedling stage was conducted to further explore variation patterns of photosynthetic apparatus structure and function across leaf positions and nitrogen treatments, with results shown in Figure 5 [Figure 5: see original paper]. The JIP-test parameters formed three well-separated clusters. Two clusters (Cluster 1 and Cluster 3) were located on the first principal component (PC1), while one cluster (Cluster 2) was located on the second principal component (PC2). Based on the physiological significance of parameters in each cluster, they corresponded to different photosynthetic processes: light absorption and capture stage (Cluster 1), electron transport and heat dissipation stage (Cluster 2), and end-chain electron transport stage (Cluster 3).

Further analysis revealed that PC1 corresponded to PSI activity, with higher values indicating higher PSI performance (high end-chain electron transport efficiency), represented by parameters such as  $\varphi_{Ro}$ ,  $\delta_{Ro}$ , and  $RE_0/RC$ . PC2 corresponded to PSII activity, with higher values indicating higher PSII performance (high light absorption, photochemical, and electron transport efficiency), represented by parameters including  $ABS/CS_m$ ,  $\varphi_{Po}$ , and  $ET_0/CS_m$ .

Clustering results from different nitrogen treatment groups showed that cluster positions changed with nitrogen application levels: the nitrogen-deficient group (N0) was mainly located in the third and fourth quadrants, the normal nitrogen group (N1) was primarily distributed between Cluster 1 and Cluster 3 in the second, third, and fourth quadrants, while the excessive nitrogen group (N2) was mainly distributed in Cluster 2 in the first quadrant. JIP-test parameter analysis revealed that compared with normal nitrogen, excessive nitrogen resulted in higher PSII and PSI performance in rape leaves, while nitrogen deficiency weakened both PSII and PSI performance, particularly in light capture and energy transfer stages. Within the same nitrogen level, cluster positions also changed with leaf position: data points representing bottom leaves (L1, darker colors) were mainly distributed in the first and fourth quadrants, while those representing top new leaves (L7, lighter colors) were located in the second and third quadrants, indicating significant differences in PSI performance (end-chain electron transfer efficiency) between top new leaves and bottom mature leaves.

Combining results from Figures 3 and 4, JIP-test parameters with high correlations to leaf biochemical parameters were all distributed within Cluster 3, which reflects end-chain electron transport performance. The vector direction of  $DF_{Total}$  pointed precisely toward the direction of increasing leaf position, consistent with results in Figure 1(c).  $DF_{Total}$  represents the driving force for electron transport to the end-chain, calculated from parameters  $PI_{ABS}$  and  $\delta_{Ro}$  (Formula (30)), which also explains why these three parameters showed small angles between their vectors and high correlations with leaf biochemical parameters in the PCA results. It can be further inferred that the comprehensive performance parameter  $DF_{Total}$  can effectively characterize vertical heterogeneity of biochemical parameters in oilseed rape canopies.

---

## 4 Conclusion

This study primarily utilized fast chlorophyll fluorescence technology to detect and evaluate vertical heterogeneity of biochemical parameters in oilseed rape canopies, yielding the following results:

1. Chlorophyll content, carotenoid content, dry matter, and water content in rape canopies at the mid-seedling stage all showed parabolic vertical distributions, while the chlorophyll-to-carotenoid ratio exhibited a distinct vertical distribution pattern, gradually decreasing with increasing leaf position and nitrogen application rate—matching the vertical distribution patterns of fluorescence parameters  $DF_{Total}$  and  $\varphi_{Ro}$ .
2. Fluorescence parameters demonstrated relatively strong evaluation capabilities for the chlorophyll-to-carotenoid ratio, chlorophyll content, and dry matter content in rape leaves. Specifically, the driving force performance parameter  $DF_{Total}$  showed highly significant positive correlations with both the chlorophyll-to-carotenoid ratio and chlorophyll content ( $r > 0.5$ ,  $P < 0.01$ ), and a significant negative correlation with dry matter content ( $r < -0.5$ ,  $P < 0.01$ ).
3. Nitrogen deficiency weakened PSII and PSI performance in rape leaves at the seedling stage, and nitrogen stress could be effectively diagnosed using fluorescence parameters such as maximum photochemical efficiency  $\varphi_{Po}$ . Significant differences in PSI performance (end-chain electron transfer efficiency) existed among leaves at different positions, and the fluorescence parameter  $DF_{Total}$  could effectively characterize vertical heterogeneity of canopy biochemical parameters.

These findings demonstrate that chlorophyll fluorescence technology can serve as an effective alternative method for evaluating vertical heterogeneity of crop biochemical parameters, providing new insights and technical support for achieving high-throughput phenotyping-based crop growth monitoring. Future research will further characterize biochemical parameters within rape canopies at dif-

ferent growth stages to enable monitoring of biochemical parameter vertical distribution throughout the entire growth period.

---

## References

- [1] ABDALLAH M, DUBOUSSET L, MEURIOT F, et al. Effect of mineral sulphur availability on nitrogen and sulphur uptake and remobilization during the vegetative growth of *Brassica napus* L[J]. *Journal of Experimental Botany*, 2010, 61(10): 2635-2646.
- [2] PAO Y, CHEN T, MOUALEU-NGANGUE D, et al. Environmental triggers for photosynthetic protein turnover determine the optimal nitrogen distribution and partitioning in the canopy[J]. *Journal of Experimental Botany*, 2019, 70(9): 2419-2434.
- [3] HIKOSAKA K. Optimality of nitrogen distribution among leaves in plant canopies[J]. *Journal of Plant Research*, 2016, 129(3): 299-311.
- [4] ADACHI S, YOSHIKAWA K, YAMANOUCHI U, et al. Fine mapping of carbon assimilation rate 8, a quantitative trait locus for flag leaf nitrogen content, stomatal conductance and photosynthesis in rice[J]. *Frontiers in Plant Science*, 2017, 8: ID 60.
- [5] WANG J, WANG Z, HUANG W, et al. The vertical distribution characteristic and spectral response of canopy nitrogen in different layer of winter wheat[J]. *Journal of Remote Sensing*, 2004, 8(4): 309-316.
- [6] YE X, ZOU Y, YU Z, et al. Correlation between nitrogen vertical distribution and spectral characteristics of flue-cured tobacco[J]. *Transactions of the CSAM*, 2013, 44(5): 219-225.
- [7] FENG W, GUO B, WANG Z, et al. Measuring leaf nitrogen concentration in winter wheat using double-peak spectral reflection remote sensing data[J]. *Field Crops Research*, 2014, 159: 43-52.
- [8] LI F, MISTELE B, HU Y, et al. Remotely estimating aerial N status of phenologically differing winter wheat cultivars grown in contrasting climatic and geographic zones in China and Germany[J]. *Field Crops Research*, 2012, 138: 21-32.
- [9] STAGAKIS S, MARKOS N, SYKIOTI O, et al. Tracking seasonal changes of leaf and canopy light use efficiency in a *Phlomis fruticosa* Mediterranean ecosystem using field measurements and multi-angular satellite hyperspectral imagery[J]. *ISPRS Journal of Photogrammetry and Remote Sensing*, 2014, 97: 138-151.
- [10] ZHANG Q, CHEN J M, JU W, et al. Improving the ability of the photochemical reflectance index to track canopy light use efficiency through differentiating sunlit and shaded leaves[J]. *Remote Sensing of Environment*, 2017, 194: 1-15.

- [11] STIRBET A, LAZÁR D, GUO Y, et al. Photosynthesis: Basics, history and modelling[J]. *Annals of Botany*, 2020, 126(4): 511-537.
- [12] GUO Y, TAN J. Recent advances in the application of chlorophyll a fluorescence from photosystem II[J]. *Photochemistry and Photobiology*, 2015, 91(1): 1-14.
- [13] GUANTER L, ZHANG Y, JUNG M, et al. Global and time-resolved monitoring of crop photosynthesis with chlorophyll fluorescence[J]. *Proceedings of the National Academy of Sciences*, 2014, 111(14): 1327-1333.
- [14] PAPAGEORGIOU G. Chlorophyll a fluorescence: A signature of photosynthesis[M]. Dordrecht: Springer, 2004.
- [15] MAGYAR M, SIPKA G, KOVÁCS L, et al. Rate-limiting steps in the dark-to-light transition of Photosystem II-revealed by chlorophyll-a fluorescence induction[J]. *Scientific Reports*, 2018, 8(1): 1-9.
- [16] SHRESTHA S, BRUECK H, ASCH F. Chlorophyll index, photochemical reflectance index and chlorophyll fluorescence measurements of rice leaves supplied with different N levels[J]. *Journal of Photochemistry and Photobiology B: Biology*, 2012, 113: 7-13.
- [17] LIU R, WANG Y, CHEN B, et al. Effects of nitrogen levels on photosynthesis and chlorophyll fluorescence characteristics under drought stress in cotton flowering and boll-forming stage[J]. *Acta Agronomica Sinica*, 2008, 34(4): 675-683.
- [18] FENG W, HE L, ZHANG H-Y, et al. Assessment of plant nitrogen status using chlorophyll fluorescence parameters of the upper leaves in winter wheat[J]. *European Journal of Agronomy*, 2015, 64: 78-87.
- [19] LARBI A, VÁZQUEZ S, EL-JENDOUBI H, et al. Canopy light heterogeneity drives leaf anatomical, ecophysiological, and photosynthetic changes in olive trees grown in a high-density plantation[J]. *Photosynthesis Research*, 2015, 123(2): 141-155.
- [20] KAUTSKY H, HIRSCH A. Neue versuche zur kohlenensäureassimilation[J]. *Naturwissenschaften*, 1931, 19(48): 964-964.
- [21] STRASSER R J, TSIMILLI-MICHAEL M, SRIVASTAVA A. Analysis of the chlorophyll a fluorescence transient[M]. Dordrecht: Springer, 2004.
- [22] STIRBET A. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient[J]. *Journal of Photochemistry and Photobiology B: Biology*, 2011, 104(1-2): 236-257.
- [23] TSIMILLI-MICHAEL M, EGGENBERG P, BIRO B, et al. Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and *Azospirillum* and *Rhizobium* nitrogen-fixers on the photosynthetic activity of alfalfa, probed by

the polyphasic chlorophyll a fluorescence transient OJIP[J]. *Applied Soil Ecology*, 2000, 15(2): 169-182.

[24] ABDI H, WILLIAMS L J. Principal component analysis[J]. *Wiley Interdisciplinary Reviews: Computational Statistics*, 2010, 2(4): 433-459.

[25] HUANG W, YANG Q, PU R, et al. Estimation of nitrogen vertical distribution by bi-directional canopy reflectance in winter wheat[J]. *Sensors*, 2014, 14(11): 20347-20358.

[26] MERZLYAK M N, GITELSON A A, CHIVKUNOVA O B, et al. Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening[J]. *Physiologia Plantarum*, 1999, 106(1): 135-141.

[27] ZHOU X, HUANG W, ZHANG J, et al. A novel combined spectral index for estimating the ratio of carotenoid to chlorophyll content to monitor crop physiological and phenological status[J]. *International Journal of Applied Earth Observation and Geoinformation*, 2019, 76: 128-142.

[28] DINÇ E, CEPPI M G, TÓTH S Z, et al. The chl a fluorescence intensity is remarkably insensitive to changes in the chlorophyll content of the leaf as long as the chl a/b ratio remains unaffected[J]. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 2012, 1817(5): 770-776.

*Note: Figure translations are in progress. See original paper for figures.*

*Source: ChinaXiv – Machine translation. Verify with original.*