Postprint: Estimation of Chlorophyll Content in Winter Wheat Under Low Temperature Stress Based on Spectral Transformation

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Abstract

In recent years, freeze damage has become one of the agrometeorological disasters affecting winter wheat regions in China, and timely, rapid, and accurate acquisition of winter wheat chlorophyll content is of utmost significance for monitoring the occurrence of freeze damage in winter wheat. This study conducted low temperature stress experiments on two winter wheat varieties at the jointing stage, subjecting them to stress treatments of -6 °C for 4 h, 8 h, and 12 h, measured canopy spectral reflectance, and applied 15 typical transformation processes to raw spectral data. PLSR models for winter wheat chlorophyll content under different spectral transformations were analyzed and compared to screen the optimal spectral transformation method capable of characterizing winter wheat chlorophyll content under low temperature stress. The results indicated that with prolonged low temperature stress duration, chlorophyll content in both winter wheat varieties exhibited a decreasing trend, and as the number of days after low temperature stress increased, differences between each treatment and the control gradually diminished. At 5 d after stress, reflectance in the near-infrared region increased substantially and rose with extended time after low temperature stress; in the visible light region, differences were not significant in the short term. At 10 d, 20 d, and 35 d after stress, the yellow and red bands gradually became horizontal, while differences in near-infrared region reflectance gradually narrowed, and spectral reflectance in the visible light region exhibited varying degrees of increase. Following 15 typical transformation processes on raw spectral data, it was found that transformations such as reciprocal, logarithmic, power, and square root of the raw spectrum were difficult to improve correlation with chlorophyll content, and the modeling effects were relatively poor. Except for the first-order differential of raw spectrum logarithm (T6), chlorophyll content diagnostic models from other differential transformation processes were all superior to those from the raw spectrum. Comprehensively considering model calibration, validation effects, optimal number of factors, and magnitude of relative analysis error, the second-order differential transformation process (T15) yielded chlorophyll content calibration model R2 and RMSE values of 0.930 and 0.340, respectively, and validation model R2 of 0.753, demonstrating that spectral transformation data based on T15 can achieve accurate estimation of chlorophyll content under low temperature stress, making it the optimal spectral transformation method.

Full Text

Preamble

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Using Spectral Transformation Processes to Estimate Chlorophyll Content of Winter Wheat Under Low Temperature Stress

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Abstract: Chlorophyll content is a vital indicator of photosynthetic capacity and crop growth status. In recent years, freeze injury has become a major agricultural meteorological disaster affecting winter wheat regions in China. Timely, rapid, and accurate acquisition of winter wheat chlorophyll content is extremely important for monitoring freeze injury occurrence. This study conducted low-temperature stress experiments on two winter wheat varieties at the jointing stage, subjecting them to -6 °C treatments for 4 h, 8 h, and 12 h. Canopy spectral reflectance was measured and 15 typical transformation methods were applied to the raw spectral data. Partial least squares regression (PLSR) models for winter wheat chlorophyll content under different spectral transformations were analyzed and compared to identify the optimal spectral transformation method for characterizing chlorophyll content under low-temperature stress. Results showed that chlorophyll content in both varieties decreased with prolonged low-temperature stress duration. As days after stress increased, differences between treatments and control gradually diminished. Five days after stress, reflectance in the near-infrared region increased substantially and continued to rise with time. In the visible region, differences were not significant in the short term. At 10 d, 20 d, and 35 d after stress, the yellow and red bands gradually leveled off while differences in near-infrared reflectance narrowed, with visible region reflectance showing varying degrees of increase. Among the 15 transformation methods, reciprocal, logarithmic, power, and square root transformations of raw spectra failed to improve correlation with chlorophyll content and yielded poor modeling results. Except for the first derivative of logarithmic transformation (T6), all differential transformation models outperformed the raw spectrum. Comprehensive evaluation of model calibration, validation performance, optimal factor number, and relative percent deviation indicated that the second derivative transformation (T15) achieved the best results, with R^2 and RMSE of 0.930 and 0.340 for the calibration model, and R^2 of 0.753 for the validation model. This demonstrates that spectral data based on T15 transformation can accurately estimate chlorophyll content under low-temperature stress and represents the optimal spectral transformation approach.

Keywords: Winter wheat; Chlorophyll content; Spectral transformation; Low temperature stress; Partial least squares regression (PLSR)

Introduction

Chlorophyll content is a crucial indicator for assessing crop photosynthetic capacity and growth status. In recent years, freeze injury at the jointing stage has become a major agricultural meteorological disaster affecting winter wheat regions in northern China. Although global climate has warmed since the 1980s, freeze injury has not diminished. Climate warming has led to a decline in the proportion of winter-planted wheat varieties and a rise in spring-planted varieties. With increasingly warm autumns, the problem of wheat overgrowth has worsened, reducing cold resistance. Moreover, climate change has become more unstable with increased extreme weather events, implying that the risk of wheat freeze injury persists. Shanxi Province has suffered freeze injury with affected areas reaching an estimated 0.26 million hectares, severely impacting wheat growth and development and ultimately affecting yield. Rapid and accurate estimation of winter wheat chlorophyll content is therefore meaningful for resisting freeze injury occurrence.

Conventional methods for measuring chlorophyll content are complex and time-consuming. Developing rapid, non-destructive chlorophyll diagnosis technology can provide an effective approach for monitoring winter wheat freeze injury. Previous studies have demonstrated that spectral transformations such as derivatives, logarithms, and normalization can improve correlations with soil organic matter. Researchers have analyzed correlations between over ten spectral indices and chlorophyll content in crops like corn and winter wheat, constructing estimation models. Studies have established regression models between winter wheat canopy hyperspectral sensitive indices and individual plant yield components to build a diagnostic index system for late frost damage. Others have used principal component analysis to achieve severity inversion of winter wheat freeze injury. First derivative processing of raw spectra has been used to analyze response characteristics of winter wheat canopy hyperspectral data to low-temperature stress. Research on sweet orange trees found that applying different spectral transformations after wavelet denoising in PLSR modeling achieved model accuracy of 0.8713 for full growth period chlorophyll monitoring. While these studies have estimated chlorophyll content using various methods and achieved results with spectral transformation in other crops and research fields, few have comprehensively applied multiple spectral transformation methods to winter wheat under low-temperature stress.

This study conducted field plot experiments on winter wheat at the jointing stage under low-temperature stress, using chlorophyll content as the dependent variable. By applying 15 transformation methods to raw spectra and comparing correlations between different transformed spectra and chlorophyll content, we constructed PLSR models to evaluate their feasibility comprehensively. The objective was to identify optimal spectral transformation methods for accurately and rapidly retrieving winter wheat chlorophyll content, providing a theoretical basis for applying hyperspectral technology in winter wheat freeze injury monitoring.

1. Materials and Methods

1.1 Experimental Materials

The experiment was conducted from October 2015 to June 2016 at the Scientific Observation and Experimental Station of Crop Cultivation and Farmland Conservation in the North China Loess Plateau Region, Ministry of Agriculture, Shanxi Agricultural University. Two winter wheat varieties were used: 'Linmai 7006' (semi-winter type) and 'Jintai 182' (winter type).

1.2 Experimental Design

The experiment consisted of two plots, each 6 m × 6 m, with row spacing of 20 cm. Sowing occurred on October 2, 2015, with conventional fertilization and field management following standard high-yield practices. Within each plot, three uniformly growing winter wheat areas were randomly selected for low-temperature stress treatments on April 15-16, 2016 (jointing stage). A custom mobile refrigeration unit simulated natural cooling processes. Each treatment area measured 60 cm × 60 cm, with a treatment temperature of -6 °C and durations of 4 h, 8 h, and 12 h. The control measurement was taken at 15 °C atmospheric temperature, with three replications.

1.3 Canopy Spectral Measurement

Canopy spectral reflectance was measured using an ASD FieldSpec 3 spectroradiometer (ASD, USA) at 5 d, 10 d, 20 d, and 35 d after low-temperature stress treatment. The spectral sampling interval was 1.4 nm with 3 nm resolution for 350–1,000 nm, and 2 nm sampling interval with 10 nm resolution for 1,000–2,500 nm. Measurements were taken between 10:00–14:00 under clear, windless conditions. Due to atmospheric and environmental influences, the 350–1,600 nm band was selected for analysis.

During measurement, the sensor probe was maintained perpendicular to the ground at approximately 1.2 m above the canopy. Three observation points were selected per treatment, with ten measurements per point averaged to obtain the spectral reflectance data. White reference calibration was performed regularly to obtain relative reflectance values.

1.4 Chlorophyll Content Measurement

Synchronized with spectral measurements, functional leaves were collected from each treatment plot, immediately sealed in bags, with three replications per treatment. In the laboratory, leaves were cut and mixed, then extracted with 80% acetone for 24 h in darkness. Optical density was measured at 663 nm and 645 nm to calculate chlorophyll content.

1.5 Spectral Transformation Methods

Based on previous research, 15 typical mathematical transformation methods were selected for winter wheat canopy spectral data transformation, as shown in Table 1 .

Table 1 Specific algorithm of fifteen spectral transformations

Abbreviation	Algorithm	Abbreviation	Algorithm	Abbreviation	Algorithm
T0	R	T5	R	T10	(lgR)
T1	1/R	T6	(lgR)	T11	(1/R)
T2	lgR	T7	(R^2)	T12	(1/R)
T3	R^2	T8	($\sqrt{}$R)	T13	(1/lgR)
T4	$\sqrt{}$R	T9	R	T14	(1/lgR)
				T15	R

Note: R is the spectral reflectance value at wavelength (nm).

1.6 Model Development

1.6.1 Calibration and Validation Set Partition A total of 96 chlorophyll content samples were measured. After outlier removal, samples were randomly divided in a 2:1 ratio, with two-thirds (64 samples) used as the calibration set and one-third (32 samples) as the validation set.

1.6.2 PLSR Model Construction and Application Partial least squares regression (PLSR) integrates advantages of principal component analysis, multiple linear regression, and correlation analysis. Leave-one-out cross-validation was used to determine the optimal number of latent variables. Model performance was evaluated using coefficient of determination (R^2), root mean square error (RMSE), and relative percent deviation (RPD). Higher R^2 and RPD values with lower RMSE indicate better model feasibility and robustness.

1.7 Statistical Analysis

ViewSpec Pro software was used for raw hyperspectral data preprocessing. Excel 2007 performed data transformations. Matlab R2010a conducted PLS analysis. Origin 8.0 processed, analyzed, and plotted relevant data.

2. Results

2.1 Chlorophyll Content Variation Under Low-Temperature Stress

Analysis of chlorophyll content changes in both varieties after low-temperature stress (Figure 1 [Figure 1: see original paper]) revealed a decreasing trend with prolonged stress duration. Five days after stress, significant differences were observed between the control and 12 h stress treatment for both 'Linmai 7006' and 'Jintai 182' , while other treatments showed lower chlorophyll content than the control without significant differences. This indicates that low-temperature stress affected leaf chlorophyll synthesis, with more pronounced effects from longer stress durations in the short term. Overall, chlorophyll content reduction increased with stress duration. At 10 d, 20 d, and 35 d after stress, differences among treatments were not significant, but the gap between stressed and control plants gradually narrowed as the crop exhibited self-repair capabilities with advancing growth stages.

2.2 Characteristics of Raw Canopy Spectra Under Low-Temperature Stress

Figure 2 [Figure 2: see original paper] shows spectral variation characteristics for different varieties after low-temperature stress. Canopy spectral curves followed consistent patterns across treatments. Except at 35 d after stress, reflectance differences were evident during other periods, showing similar variation patterns. With extended low-temperature duration, spectral reflectance exhibited regular differential changes. Five days after stress (Figure 2a), decreasing chlorophyll content caused visible region reflectance to increase to varying degrees, with the 12 h treatment (S3) reaching the highest values at green peaks and red valleys. However, differences were not obvious in this region due to relatively low reflectance (<0.1). In contrast, near-infrared region differences were significant, with substantial reflectance increases compared to the control, particularly for the 8 h treatment (S2) which reached 0.38. Ten days after stress (Figure 2b), visible region reflectance remained low while near-infrared differences persisted, with S2 showing the highest reflectance at 0.42. Twenty days after stress (Figure 2c), the visible region flattened, red valleys became indistinct, green peaks weakened, and reflectance stayed low. Near-infrared differences narrowed, with the 4 h treatment (S1) showing the highest reflectance at 0.36, overall lower than the previous period. Thirty-five days after stress, the visible region flattened with elevated red valleys and low reflectance, while near-infrared differences continued narrowing, indicating recovery with advancing growth stages. Figures 2e–2h show that 'Jintai 182' exhibited similar spectral response patterns to 'Linmai

7006' , but with higher canopy reflectance values overall. Chlorophyll content was closely related to canopy spectral reflectance changes. In the 400–700 nm region, strong absorption by chlorophyll a, b, and other pigments resulted in low reflected and transmitted light, creating two absorption valleys. When chlorophyll content decreased, reflectance increased, making absorption valleys less distinct. Relying solely on raw canopy spectra would hinder spectral information extraction for chlorophyll content and reduce monitoring model accuracy, necessitating spectral preprocessing.

2.3 Spectral Reflectance After Transformation

Applying the mathematical transformations from Table 1 produced the results shown in Figure 3 [Figure 3: see original paper]. Compared to raw spectral data (T0), transformations T2, T4, T13, and T14 showed no obvious curve characteristic changes. T1 and T3 exhibited significant curve changes, while T5, T6, T7, T8, T9, T10, T11, T12, and T15 demonstrated significant effects in improving signal-to-noise ratio and refining spectral information. This indicates that differential transformations can eliminate linear or near-linear noise effects, making spectral characteristic information more apparent.

2.4 Correlation Analysis Between Chlorophyll Content and Transformed Spectra

Extreme weather conditions (e.g., freeze injury) prevent winter wheat roots from absorbing water, severely disrupting cellular membrane systems and rupturing chloroplasts, leading to chlorophyll degradation and abrupt chlorophyll content changes. Real-time monitoring of these changes provides guidance for resisting freeze injury. Correlation analysis reliably examines relationships between variables. Analysis of correlations between transformed spectral data and chlorophyll content after low-temperature stress (Figure 4 [Figure 4: see original paper]) showed that compared to raw spectra (T0), transformations T1, T2, T3, T4, T13, and T14 did not improve correlations, while T5, T6, T7, T8, T9, T10, T11, T12, and T15 significantly enhanced correlations, all exceeding 0.7. Comparing Figures 3 and 4 reveals that spectra with larger variation amplitudes also showed greater correlation improvements, with correlation coefficients increasing markedly, particularly for differential transformations. This demonstrates that differential transformation of raw spectra significantly improves correlation with chlorophyll content.

2.5 PLSR Model Development

2.5.1 Determination of Optimal Latent Variables Using canopy raw spectral reflectance and 15 transformed spectra as independent variables and chlorophyll content as the dependent variable, leave-one-out cross-validation determined the optimal number of latent variables for regression models, as shown in Figure 5 [Figure 5: see original paper]. Selecting the optimal factor number is prerequisite for accurate spectral feature extraction and stable, precise model construction. Too few or too many latent variables affect prediction accuracy. This study used the balance point of RMSECV variation curves for different spectral transformations as the optimal factor number: T0, T1, T2, T3, T4, T7, T9, T13, and T14 required 4 factors; T5, T6, T11, and T12 required 2 factors; T8 required 5 factors; and T10 and T15 required 3 factors.

2.5.2 Winter Wheat Chlorophyll Content Estimation Using PLSR PLSR was applied to raw and transformed canopy spectra for comprehensive regression analysis of calibration and validation sets to identify optimal spectral transformations and corresponding monitoring models. Results are presented in Table 2 .

When model accuracy is similar, fewer independent variables increase application value. Optimal factor number, correlation coefficient, and RPD are also important reference indicators. Table 2 shows that different spectral transformations significantly affected model construction and prediction. Except for T1, T2, T3, T4, T6, and T14, all other transformations achieved higher monitoring accuracy (R^2) than raw spectra (R^2 = 0.776), indicating that spectral transformation can improve monitoring precision for chlorophyll content under low-temperature stress. Although T8 and T13 models outperformed T0, their RPD values were below 1.4, indicating poor predictive ability. The T8 calibration model required more latent variables (Fn = 5), increasing model complexity and risk of overfitting. T13 showed lower correlation with chlorophyll content, reducing model stability. Balancing accuracy and complexity, transformations T5, T7, T9, T10, T11, T12, and T15 optimized chlorophyll monitoring models and improved diagnostic accuracy. Particularly, models built with T9 and T15 achieved high precision with fewer latent variables. Overall, T15 demonstrated superior accuracy, RPD, and correlation coefficient compared to T9, with fewer optimal factors, making T15 the optimal spectral transformation method. In summary, T15 transformation can improve diagnostic precision for chlorophyll content under freeze injury and should be considered the preferred spectral data transformation method, with T9 as the secondary option.

3. Discussion and Conclusion

Chlorophyll is a primary factor affecting crop photosynthesis, and its content directly influences crop metabolism and yield formation. This study analyzed chlorophyll content variation patterns in different winter wheat varieties under low-temperature stress. Five days after stress, all treatments showed lower chlorophyll content than the control, with more pronounced decreases as stress duration increased. This occurred because low-temperature stress damaged leaf internal structures. By 35 days after stress, the impact diminished as crops possess self-regulation and repair capabilities, consistent with previous findings.

Original spectral curves showed consistent patterns across treatments. Five days after stress, visible region reflectance increased to varying degrees due to reduced chlorophyll content weakening pigment absorption. Near-infrared region differences were significant, with substantial reflectance increases compared to the control. Spectral patterns at 10 d and 20 d were similar. By 35 d, the visible region flattened with elevated red valleys, and near-infrared differences narrowed as chlorophyll content partially recovered.

However, analyzing raw spectral characteristics alone is insufficient for freeze injury monitoring. This study applied 15 mathematical transformations to canopy spectral data and compared PLSR model accuracies to select optimal methods. Research indicates differential processing is important for reducing background and noise effects. This study found that differential transformations significantly altered spectra, increasing inter-band differences and improving correlations with chlorophyll content. PLSR integrates advantages of principal component analysis, multiple linear regression, and correlation analysis, providing guidance for quantitative relationships between full-band spectra and chlorophyll content under low-temperature stress.

Both calibration and validation results demonstrated that differential transformation improved diagnostic accuracy for chlorophyll content models under low-temperature stress, consistent with previous research. While first derivative processing performed well, comprehensive evaluation of diagnostic accuracy, model complexity, optimal factor number, and RPD indicated that second derivative transformation (T15) of raw spectra achieved the best performance ($R^2 = 0.930$, RMSE = 0.340, RPD = 3.807) with fewer latent variables (Fn = 3). This may be because chlorophyll content under low-temperature stress is influenced by variety, climate, and cultivation conditions. Transformations with lower correlation required more latent variables to achieve high PLSR accuracy, but excessive factors increase complexity and cause overfitting, reducing application value.

Although T15 transformation yielded high-precision models for chlorophyll content under low-temperature stress, model construction and validation were conducted under experimental conditions. Further research is needed on chlorophyll content estimation under low-temperature stress across different growth stages, years, regions, varieties, and cultivation methods to achieve more precise freeze injury monitoring.

In conclusion: (1) Compared with raw spectra, transformations not involving differential processing (reciprocal, logarithmic, power, square root) failed to improve correlation with chlorophyll content and produced poor models. (2) The second derivative transformation (T15) performed best, enabling accurate spectral estimation of chlorophyll content. First derivative transformation (T9) can be considered as an alternative method. These results provide theoretical basis and technical reference for chlorophyll content estimation and spectral data preprocessing.

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