Postprint: Visible/Near-Infrared Spectroscopic Inversion of Malondialdehyde Content in Juncao Based on Deep Convolutional Generative Adversarial Network

Authors: Dapeng Ye, Chen Chen, Li Huilin, Lei Yingxiao, Weng Haiyong, Qu Fangfang

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Abstract

[Purpose/Significance] Juncao is a perennial herbaceous plant that can be used as feed and biomass energy, and its cultivation in temperate zones requires overcoming winter survival challenges. Low temperature stress adversely affects the growth and development of juncao. Malondialdehyde (MDA) content serves as a powerful diagnostic indicator for diagnosing low temperature stress status in juncao, and using spectral technology to invert MDA content enables rapid and non-destructive assessment of juncao growth dynamics, providing a reference for juncao breeding and low temperature stress diagnosis.

[Methods] This study was based on six varieties of juncao plants, with low temperature stress and normal temperature control groups established to acquire Visible/Near Infrared Spectrum (VIS/NIR) data and leaf MDA content information during the juncao seedling stage, analyzing the increasing trend of both juncao MDA content and its spectral reflectance under low temperature stress conditions; to improve model detection performance, an improved one-dimensional Deep Convolutional Generative Adversarial Networks (DCGAN) was proposed for sample quantity augmentation, and MDA spectral quantitative detection models were established based on Random Forest (RF), Partial Least Squares Regression (PLSR), and Convolutional Neural Networks (CNN) algorithms.

[Results and Discussion] DCGAN can optimize model reliability and MDA detection accuracy, and the DCGAN combined with RF model can achieve the best detection performance, with a prediction set coefficient of determination Rp2 of 0.7922, root mean square error of 2.4063, and Residual Predictive Deviation (RPD) of 2.1937.

[Conclusion] This study utilized DCGAN for sample quantity augmentation, which can significantly improve the inversion accuracy and prediction performance of spectral data-based models for juncao MDA content.

Full Text

Preamble

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Visible/NIR Spectral Inversion of Malondialdehyde Content in JUNCAO Based on Deep Convolutional Generative Adversarial Network

YE Dapeng[1,2], CHEN Chen[1,2], LI Huilin[1,2], LEI Yingxiao[3], WENG Haiyong[1,2], QU Fangfang[1,2*]

[1]College of Mechanical and Electrical Engineering, Fujian Agriculture and Forestry University, Fuzhou, Fujian
[2]Key Laboratory of Agricultural Information Perception Technology, Fujian Province, Fuzhou, Fujian
[3]College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou, Fujian

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Abstract:
[**Objective/Significance**] JUNCAO is a perennial herbaceous plant utilized as feedstock and biomass energy, but its cultivation in temperate zones requires overcoming overwintering challenges. Low-temperature stress adversely affects JUNCAO growth and development. Malondialdehyde (MDA) content serves as a powerful diagnostic indicator for low-temperature stress status in JUNCAO. Using spectral technology to invert MDA content enables rapid, non-destructive assessment of JUNCAO growth dynamics, providing valuable references for JUNCAO breeding and low-temperature stress diagnosis. [**Methods**] This study employed six JUNCAO varieties, establishing both low-temperature stress and normal-temperature control groups. Visible/near-infrared (VIS/NIR) spectral data and leaf MDA content information were acquired during the seedling stage. Under low-temperature stress, both MDA content and spectral reflectance showed corresponding increasing trends. To enhance model detection performance, an improved one-dimensional deep convolutional generative adversarial network (DCGAN) was proposed for sample augmentation, combined with partial least squares regression (PLSR) and convolutional neural network (CNN) algorithms to establish VIS/NIR spectral quantitative detection models. [**Results and Discussion**] The DCGAN model optimized model reliability and detection accuracy, with sample augmentation improving performance. The DCGAN-based approach achieved the best detection results, with

a prediction set coefficient of determination (Rp^2) and root mean square error of prediction (RMSEP) of [MATH_0]. **Conclusion** This study demonstrates that using DCGAN for sample augmentation significantly improves the inversion accuracy and predictive performance of spectral-data-based models for JUNCAO MDA content.

Keywords: JUNCAO; visible/near-infrared spectroscopy; deep convolutional generative adversarial network; low-temperature stress; machine learning
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Introduction

JUNCAO refers to herbaceous plants used as cultivation substrates for edible and medicinal fungi. With characteristics of high biomass and nutrient content, JUNCAO holds tremendous application value in feed, biomass energy, and material development. Currently, JUNCAO cultivation technology developed in China has been promoted to numerous countries and regions worldwide, playing a significant role in promoting global economic development [1-4]. Cold damage under low-temperature stress is a global natural disaster that particularly adversely affects agricultural production [5,6] and represents a critical factor influencing JUNCAO geographical distribution and productivity [7-9]. Therefore, dynamic monitoring of JUNCAO health status and screening of resistant varieties are of great importance.

When plants encounter low-temperature stress, lipid peroxidation leads to protein cleavage and destruction of cell membrane systems, impairing photosynthesis and respiration, and potentially causing plant death [10]. Malondialdehyde (MDA) is a degradation product of polyunsaturated fatty acid peroxides. The more severe the low-temperature damage, the higher the MDA content in plants [11,12]. Consequently, MDA content serves as a powerful diagnostic indicator for studying plant growth dynamics. Accurate prediction of MDA content can reflect plant health status in real time. The thiobarbituric acid (TBA) method is a traditional approach for MDA determination [13], but it is time-consuming, labor-intensive, and destructive, making it difficult to conduct real-time effective detection of large-scale field crops [14]. Therefore, establishing a rapid, non-destructive, and effective MDA determination method is crucial for plant stress monitoring.

In recent years, with the development of optical sensors and machine learning technologies, visible/near-infrared spectroscopy has been widely applied in agriculture [15,16], food [17], and medicine [18] due to its rapid and non-destructive advantages. In previous studies on biochemical indicator determination, most researchers employed spectral preprocessing and modeling optimization to improve accuracy. Phuphaphud et al. [19] predicted fiber content in different parts of sugarcane using visible/near-infrared spectroscopy combined with preprocessing methods, achieving an Rp^2 of 0.81 based on partial least squares regression (PLSR). Chanda et al. [20] detected caffeine content in tea using near-infrared spectroscopy, obtaining effective wavelengths based on chemical bond response characteristics and combining preprocessing with support vector regression (SVR) and PLSR models, with the best Rp^2 reaching 0.637. Zhang Yakun et al. [21] determined soluble protein content in soybean leaves using near-infrared spectroscopy, demonstrating that Savitzky-Golay smoothing combined with successive projections algorithm (SPA) yielded optimal detection performance with a prediction set R^2 of 0.746. Although these studies achieved good results, acquiring large datasets in practical sample collection is difficult, and small datasets often affect modeling accuracy [22,23], making high-quality dataset augmentation necessary.

Radford et al. [24] proposed deep convolutional generative adversarial networks (DCGAN) in 2015 for generating high-quality images, achieving excellent results in data augmentation, image restoration, and super-resolution reconstruction [25,26]. Based on this, to explore the feasibility of using DCGAN combined with visible/near-infrared spectroscopy to invert MDA content in JUNCAO under low-temperature stress, this study selected six JUNCAO varieties, measured leaf visible/near-infrared reflectance and MDA content, employed one-dimensional DCGAN for dataset expansion, and established three quantitative detection models: one-dimensional convolutional neural network (CNN), random forest (RF), and partial least squares regression (PLSR), to achieve rapid, non-destructive detection of JUNCAO MDA content.

2. Materials and Methods

2.1 Experimental Materials

This study was conducted from October–November 2022 and March–April 2023 in the JUNCAO greenhouse at Fujian Agriculture and Forestry University (26°5'21"N, 119°14'49"E). Six typical JUNCAO varieties were selected: *Arundo donax* cv. Lvzhou No. 2 (A. donax No. 2), *Arundo donax* cv. Lvzhou No. 7 (A. donax No. 7), *Cenchrus fungigraminus* (giant JUNCAO), *Pennisetum purpureum* Schum (elephant grass), purple elephant grass (*Pennisetum purpureum* Schumab), and *Pennisetum sinese* Roxb (king grass), all provided by the National JUNCAO Engineering Technology Research Center of Fujian Agriculture and Forestry University. Plants were cultivated in soil using plug trays (upper diameter 58 mm, lower diameter 20 mm, depth 110 mm) placed in an incubator at 28°C with regular watering. When plants reached approximately 25 cm in height, each variety was evenly divided into two groups: the control group remained at 28°C, while the stress group was transferred to 4°C for treatment [27]. After 5 days, leaves from both groups were collected as experimental samples, and spectral reflectance and MDA content were measured. A total of 144 plants were sampled, with different JUNCAO varieties shown in Figure 1 [Figure 1: see original paper].

2.2 Measurement Items and Methods

2.2.1 Leaf Spectral Reflectance Acquisition A handheld spectroradiometer (ASD HandHeld 2, Malvern Panalytical, wavelength range 325–1075 nm, spectral resolution 3.5 nm) and a near-infrared spectrometer (SW2520-050-NIRA, Taiwan Ultra-Micro Optical, wavelength range 900–1700 nm, spectral resolution 9 nm) were used to collect spectral information from JUNCAO leaves in the 325–1700 nm range. Three different positions were measured for each sample, and the average was taken as the sample's spectral reflectance. The device was preheated for approximately 30 minutes before data collection and calibrated with black and white reference panels. To eliminate significant noise at the spectral ends, the 400–999 nm range (600 wavelength parameters) from the handheld spectroradiometer and the 1000–1650 nm range (99 wavelength parameters) from the near-infrared spectrometer were extracted and concatenated (699 wavelength parameters) for subsequent analysis.

2.2.2 Malondialdehyde Content Determination MDA content was measured using the thiobarbituric acid method. After spectral data collection, leaves were cut, avoiding leaf veins. Leaves from each plant were mixed, and 0.1 g was weighed into a centrifuge tube, immediately frozen in liquid nitrogen, and stored at -80°C. The specific determination method was as follows: leaves were homogenized with 1 mL extraction solution in an ice bath, then centrifuged at 8000 r/min for 10 min at 4°C. Subsequently, 0.1 mL supernatant was extracted, mixed with 0.4 mL TBA solution, heated in a water bath at 100°C for 60 min, then centrifuged at 10,000 r/min for 10 min at 25°C. Finally, 0.2 mL supernatant was taken to measure absorbance at 532 nm and 600 nm to determine MDA concentration.

2.3 Modeling Methods and Model Evaluation Criteria

2.3.1 Deep Convolutional Generative Adversarial Network The DCGAN model was implemented using Python 3.8 on a PyCharm community edition platform with PyTorch 2.0.0. The model consists of a generator and a discriminator, with the workflow shown in Figure 2 Figure 2: see original paper. The generator takes Gaussian-distributed random noise z as input to generate pseudo-data $G(z)$, while the discriminator takes both real and pseudo-data as input to output discrimination results. Upon training completion, generated samples were saved to complete data augmentation.

Since the original DCGAN was designed for image domains, this study modified the network for one-dimensional spectral data. To prevent discriminator dominance and gradient vanishing during training, which would hinder learning from original sample information, the discriminator network depth was reduced. The model structure and parameters are shown in Figure 2 Figure 2: see original paper. The generator comprises five transposed convolution layers, five activation functions, and four batch normalization layers, with the fifth layer using tanh activation and the others using ReLU, ultimately generating a 700-dimensional one-dimensional vector where the first column represents fitted MDA content and the remaining 699 columns represent spectral parameters. The discriminator includes four convolution layers, two batch normalization layers, and three LeakyReLU activation functions, with a final sigmoid activation for authenticity discrimination. The model employed the Adam optimizer with learning rates of 0.0002 for both generator and discriminator, batch size of 1, and 300 training epochs. Due to initial training instability, only the generator with the lowest loss function value after 100 epochs was saved.

2.3.2 Model Establishment The Kennard-Stone (KS) algorithm was first used to divide the original samples into a modeling set and prediction set at a 2:1 ratio, yielding 96 modeling samples and 48 prediction samples. The 96 modeling samples were then augmented through the DCGAN model to generate 384 pseudo-samples (four times the original). These pseudo-samples were randomly shuffled and sequentially added to the modeling set to form an enhanced modeling set for evaluation with different regression methods and generated sample quantities.

This study constructed PLSR, RF, and CNN models for JUNCAO MDA quantitative detection. The CNN network parameters are detailed in Table 1 . Hyperparameters were set as follows: stochastic gradient descent (SGD) optimizer, batch size of 48, 500 epochs with early stopping to prevent overfitting, and learning rate of 0.001.

2.3.3 Evaluation Metrics The coefficient of determination (R^2), root mean square error (RMSE), and residual predictive deviation (RPD) were used to evaluate model prediction performance. Generally, larger R^2 and RPD values with smaller RMSE indicate better correlation between predicted and measured values and superior prediction performance. Specific calculations are shown in equations (1)–(3):

$$R^2 = \frac{\sum_{i=1}^{n}(y_{i,p} - \bar{y}_{i,a})^2}{\sum_{i=1}^{n}(y_{i,a} - \bar{y}_{i,a})^2}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_{i,p} - y_{i,a})^2}{n}}$$

$$RPD = \frac{SD_v}{SEP}$$

where y_{i,a} and y_{i,p} are the actual and predicted MDA content values (nmol/g) for the ith sample, respectively; $\bar{y}$_{i,a} and $\bar{y}$_{i,p} are the mean actual and predicted values (nmol/g); *SD* and *SEP* are the standard deviation of actual content and standard error of prediction (nmol/g); and n is the number of samples.

3. Results and Discussion

3.1 Effects of Low-Temperature Stress on JUNCAO Leaf Spectral Reflectance and MDA Content

Figure 3 [Figure 3: see original paper] shows the average spectra and corresponding MDA content of six JUNCAO varieties after 5 days of cultivation at normal temperature (28°C) and low temperature (4°C). A noticeable offset in spectral reflectance around 1000 nm was observed, primarily due to different device parameters between the two instruments. In the visible range (400–740 nm), a strong reflection peak near 550 nm occurred because green plant leaves absorb little green light, while deep absorption valleys near 480 nm and 675 nm resulted from strong chlorophyll absorption. The near-infrared range (740–1650 nm) was mainly influenced by internal leaf structure and water content [28].

Comparative analysis revealed that stressed and control groups exhibited different spectral characteristics but consistent overall trends. Across the full wavelength range, stressed JUNCAO leaves showed significantly increased spectral reflectance compared to the control group. In the visible range, this resulted from pigment destruction caused by low-temperature stress, while in the near-infrared range, structural damage and reduced water content contributed to higher reflectance. This trend aligns with findings from other studies [29,30].

After low-temperature stress, MDA content in all varieties increased compared to control groups, demonstrating lipid peroxidation and plant damage. Different varieties yielded distinct MDA concentration gradients with uniform data distribution. Significant differences in MDA content between control and stress groups were observed for each variety ($P < 0.05$), facilitating subsequent stable quantitative modeling.

3.2 Sample Augmentation Using Deep Convolutional Generative Adversarial Network

Figure 4 Figure 4: see original paper displays the original VIS/NIR spectral reflectance of 144 JUNCAO leaf samples, while Figure 4 Figure 4: see original paper shows 384 pseudo-samples generated by the DCGAN model. The generated spectral curves retained the shape and variation trends of the original curves but were not identical, as Gaussian noise introduced in the DCGAN model increased sample diversity. The reflectance range of generated samples was smaller than that of original samples, likely because concentrated spectral reflectance values in most original samples caused the generator to produce reflectance values at other positions that were easily identified as fake by the discriminator.

Statistical characteristics of MDA content were compared between original and generated samples (Table 2). Generated sample MDA content ranged from 13.2523 to 34.5608 nmol/g, within the original sample range, with average and standard deviation decreasing by only 0.6650 and 0.9743 nmol/g, respectively. These results indicate that generated MDA content values conformed well to the original sample distribution.

Since original sample MDA content showed significant differences between control and stress groups for each variety ($P < 0.05$), with stress group values typically higher, probability density curves were established for control and stress groups separately (Figure 5 Figure 5: see original paper). The control group peaked at 17.0014 nmol/g, while the stress group peaked at 25.5955 nmol/g, with equal probability density at 20.9407 nmol/g. This threshold was used to classify generated samples, with values below 20.9407 nmol/g designated as control and above as stress. Figure 5 Figure 5: see original paper shows average spectral curves for generated control and stress groups, with the stress group exhibiting higher reflectance across most wavelengths, particularly significant at 550 nm, 1000–1300 nm, and 1450 nm, satisfactorily meeting original sample spectral characteristics.

These results demonstrate that DCGAN-generated samples exhibit high diversity while maintaining strong similarity to original samples, conforming to original distribution patterns and enabling effective data augmentation.

3.3 JUNCAO MDA Content Detection Models Based on Original and Generated Samples

Before model construction, generated samples were randomly shuffled and sequentially added to the original modeling set. PLSR, RF, and CNN models were built to evaluate DCGAN-generated data on the same prediction set. Due to long CNN training times, generated samples were added proportionally (48, 96, 192, 288, and 384 samples) when constructing the CNN regression model.

Detection results for the three models are shown in Figure 6 [Figure 6: see original paper]. Without generated samples, model performance differed significantly, with RF achieving $R^2 = 0.6967$ and RMSEP = 2.7591, CNN achieving $R^2 = 0.6729$ and RMSEP = 3.1353, and PLSR achieving only $R^2 = 0.5298$ and RMSEP = 3.6646. As generated samples were added, all three models showed an initial increase followed by decrease in R^2, while RMSEP first decreased then increased. Table 3 presents optimal training results for the three models. Compared with original sample prediction accuracy, CNN improved by up to 11.2%, RF by up to 13.7%, and PLSR by up to 42.8%, with RF achieving the best detection performance. Zhang et al. [31] estimated MDA content in pine needles using near-infrared spectroscopy, achieving $R^2 = 0.66$ after preprocessing and feature selection, compared with original $R^2 = 0.64$. In contrast, this study's data augmentation approach significantly improved all three regression models.

To visually represent prediction capability, Figure 7 [Figure 7: see original paper] shows correlation plots between predicted and measured MDA content for the three models under optimal sample sizes. The results demonstrate that DCGAN-based sample augmentation yields better accuracy and stability, with sufficient datasets contributing to optimal detection results.

Conclusion

This study investigated six JUNCAO varieties under low-temperature stress, proposing a one-dimensional DCGAN model for sample expansion combined with PLSR, RF, and CNN for quantitative MDA detection. Key findings include: (1) Low-temperature stress significantly increased JUNCAO leaf MDA content and visible/near-infrared spectral reflectance compared to control groups; (2) DCGAN effectively augmented sample size, with model accuracy initially increasing then decreasing as more samples were added; (3) All three models showed improved evaluation metrics after data augmentation, with DCGAN combined with RF achieving optimal MDA detection performance.

This research enables rapid detection of JUNCAO MDA content under low-temperature stress with small sample sizes, providing theoretical and technical support for JUNCAO breeding and low-temperature stress diagnosis.

Conflict of Interest Statement: The authors declare no conflicts of interest regarding this research.

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