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A NEAR-INFRARED REFLECTANCE SPECTROSCOPY–BASED NONDESTRUCTIVE TECHNIQUE FOR THE DETECTION OF SEED-BORNE FUNGI IN CHOY SUM (*BRASSICA CHINENSIS* VAR. *PARCHINENSIS*)

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Abstract

In the present study seed-born fungi in choy sum (*Brassica Chinensis* Var. *Parchinensis)* was detected through a near-infrared reflectance spectroscopy–based nondestructive technique. Four common species of seed-borne fungi namely, *Penicillium decumbens*, *Penicillium namyslowskii*, *Lichtheimia corymbifera*, and *Aspergillus niger* were found to grow in choy sum in southern China. A novel finding was that *P. decumbens* had the greatest inhibition of the germination of Choy sum seeds, resulting in a non-germination rate exceeding 62.8%. The existing standardized seed sterilization treatment failed to completely kill *P. decumbens*, so a way to nondestructively identify and filter out *P. decumbens*–infected seeds wereevolved is urgently needed. Near-infrared reflectance spectroscopy–based partial least squares–discriminant analysis (PLS-DA) model achieved a discrimination accuracy of 95% in on-line nondestructive detection of *P. decumbens*–infected choy sum seeds. By detailing the design and application of an innovative product, this study lays a foundation for the development of intelligent equipment, and it provides a decision-making basis for seed breeding industrialization and germplasm supervision.

Choy sum (*Brassica chinensis* var. *parachinensis*) is a special vegetable in southern China and is deeply loved by the people. Chinese farmers and scientific and technological personnel engaged in agricultural work at the grassroots level currently lack knowledge about seed treatment and do not have relevant technical skills or experience, and most individual small farmers choose to extract seeds from the fruits they harvest without spending money on conventional seed treatment. In addition, the government has not instituted relevant administrative management measures. Therefore, seed-borne diseases of choy sum have been frequently reported in recent years and have caused great losses to China's agriculture and seed industry (González *et al.* 1995, Ahmad A *et al.*2022). In the study, the near-infrared spectral characteristics and patterns of change of *P. decumbens*–infected choy sum seeds were analysed based on the characteristics of pathogenic fungi screened out of choy sum seeds, and the spectral characteristics of *P. decumbens*–infected choy sum seeds were extracted to construct a model for the identification of *P. decumbens*–infected choy sum seeds. Thus an innovative product and method that can form the basis of not only intelligent equipment to identify and sort infected seeds but also the establishment of enterprise standards for sorting seed-borne fungi, the industrialization of healthy seed breeding, and the intelligent supervision of germplasm were used(Chen *et al.*2019, Opara *et al*. 2022).

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During the present study sterile choy sum seeds, *P. decumbens*–infected choy sum seeds, a fibre-optic spectrometer (FLAME-NIR-INTSMA25 950-1650 nm), a white reflectance standard (WS-1-SL), a stand (STAGE-1), a reflection probe (QR600-7-VIS125BX), and a tunable high-power halogen tungsten light source (360-2400 nm) were used.

The system for acquisition of the near-infrared reflectance spectra of choy sum seeds was mainly composed of a light source, a reflection probe, and spectrum acquisition software. The reflection probe had a six-around-one optic fibre design at one end, which made the light shining on the measured object more uniform and suitable for single-point detection. Six fibre legs were connected to a 5-W tunable high-power halogen tungsten light source, and another fibre leg was connected to a FLAME-NIR-INTSMA25 miniature fibre-optic spectrometer (Ocean Optics USA) for optimal performance. The miniature fibre-optic spectrometer had a scanning wavelength range of 950-1650 nm and a spectral resolution of 10 nm.

Four hundred choy sum seeds were sterilized with 1% sodium hypochlorite solution, and a total of 400 choy sum seeds were infected with *P. decumbens* (Song *et al.* 2017, Wang *et al.* 2020). To avoid the influence of system instability on the experimental results, the tunable high-power halogen tungsten light source was turned on and preheated for 30 min before the spectra were collected. After the light source was stabilized and the dark and white reference spectra were collected for calibration, the sample spectra were collected. A clean filter paper was placed at the bottom of the stand tray. Then a sterile choy sum seed was picked up with a pair of tweezers and placed within the light spot on the filter paper. Next, the spectrometer operating software (Ocean View) was run. The integration time was set to 100 ms, and the average number of scans was set to 3. The seed was scanned from 950 nm to 1650 nm, and its spectrum was stored in the computer. After the sterile choy sum seeds were tested, the filter paper was replaced, and the tweezers were sterilized. A choy sum seed infected with *P. decumbens* was picked up with the tweezers and placed within the light spot on the filter paper, and the above spectrum acquisition steps were repeated.

To explore whether the length of time after infection affected the spectra of choy sum seeds, the near-infrared reflectance spectra of the choy sum seeds were collected on the 3rd, 5, 7, 9, 11, 13, 15, 17 and 19th days after the choy sum seeds were infected with *P. decumbens*.

Affected by the external detection environment and instrument performance, the original spectra had much noise at both ends, which would affect the modelling results, so the raw spectra had to be preprocessed. The original spectra of the 400 sterile choy sum seeds and the 400 seeds collected on the 13th day after infection with *P. decumbens* were first preprocessed by Savitzky-Golay (SG) convolution smoothing and then by standard normal variate (SNV) transformation (Ding *et al*. 2020, Araújo *et al.* 2001). The preprocessing results are shown in Fig. 1. Due to space limitations and the independence of the discrimination accuracy of the model from the length of time after infection, the following descriptions are based on the data collected on the 13th day after infection.

Figure 1d showed the average spectra obtained after SNV transformation. There was a significant difference between the reflection spectra of the *P. decumbens*–infected choy sum seeds and the sterile choy sum seeds. Specifically, the reflectance spectrum of the *P. decumbens*–infected choy sum seeds showed an increasing trend in the 950-1100-nm range, while the reflectance spectrum of the sterile choy sum seeds was quite flat in this range. Based on this distinct difference between the reflectance spectra of the *P. decumbens*–infected choy sum seeds and the sterile choy sum seeds, a built a model for the rapid identification of *P. decumbens*–infected choy sum seeds based on SNV-transformed spectra was built.

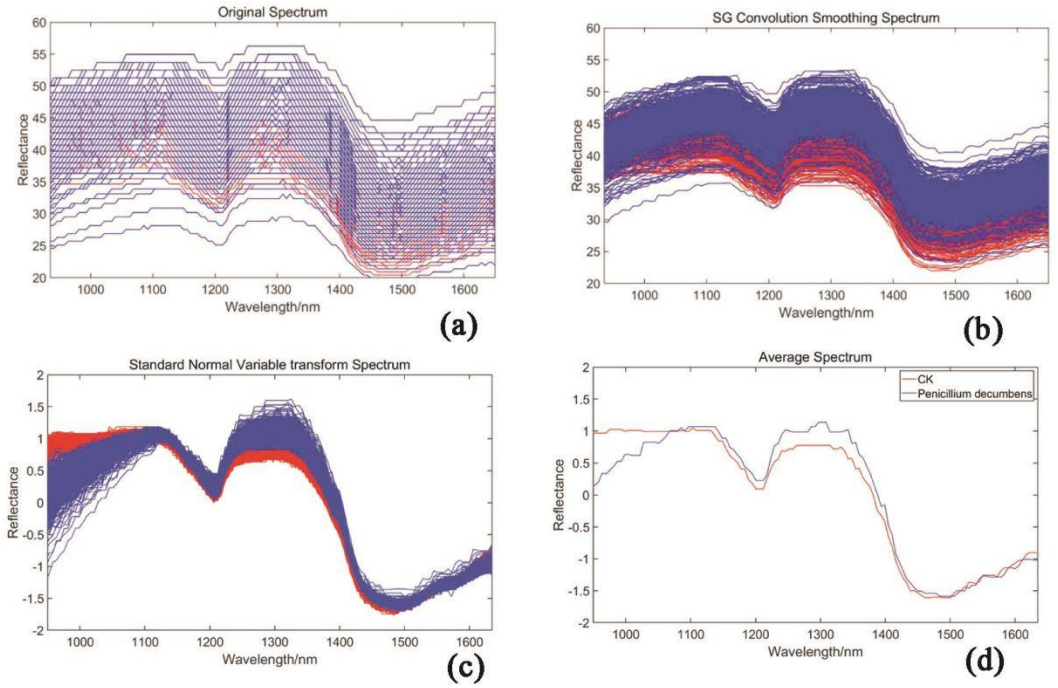


Fig. 1a-d: (a). Original spectra, (b). SG-smoothed spectra, (c) SNV-transformed spectra, (d) Average spectra.

Based on the reflectance spectra of 800 choy sum seed samples (including 400 sterile seeds and 400 seeds infected with *P. decumbens*) in the 950-1650 nm range, a prediction model for identification of *P. decumbens*–infected choy sum seeds were established by partial least squares–discriminant analysis (PLS-DA) and was hereinafter called the PLS-DA model (Fu *et al*. 2007, Daneshvar *et al*. 2015, Ding *et al*. 2020). The Kennard–Stone algorithm was used to divide the 800 samples into a calibration set (600 samples, including 300 *P. decumbens*–infected choy sum seeds and 300 sterile choy sum seeds) and a validation set (200 samples, including 100 *P. decumbens*–infected choy sum seeds and 100 sterile choy sum seeds) at a ratio of 3:1. Figure 2 showed the PLS-DA model results. There were three misidentified samples in the calibration set (two *P. decumbens*–infected choy sum seeds and one sterile choy sum seed), so the discrimination accuracy in the calibration set was 99%. There were three misidentified samples in the validation set (three sterile choy sum seeds), so the discrimination accuracy of the validation set was 99%. The overall discrimination accuracy of the model was 99%.

The length of time after infection did not affect the accuracy of the PLS-DA model. Table 1 showed the accuracy of the PLS-DA model at the 3rd, 5, 7, 9, 11, 13, 15, 17 and 19th days after the choy sum seeds were infected with *P. decumbens*.

A total of 120 untreated choy sum seed samples were numbered from 1-120, as shown in Fig. 3. The near-infrared reflectance spectra of each sample were collected in sequence, totalling 120 spectra. The original spectra were preprocessed by SNV transformation and then input into the PLS-DA model. The PLS-DA model showed that 20 samples were infected by *P. decumbens*. To verify the accuracy of the PLS-DA model, each of the 120 choy sum seed samples was individually cultured on a Pichia adenine dropout (PAD) plates, and the number of *P. decumbens*–infected seeds was counted (judged according to the colour and shape of *P. decumbens*) and then compared with that predicted by the PLS-DA model (Li *et al*. 2010).

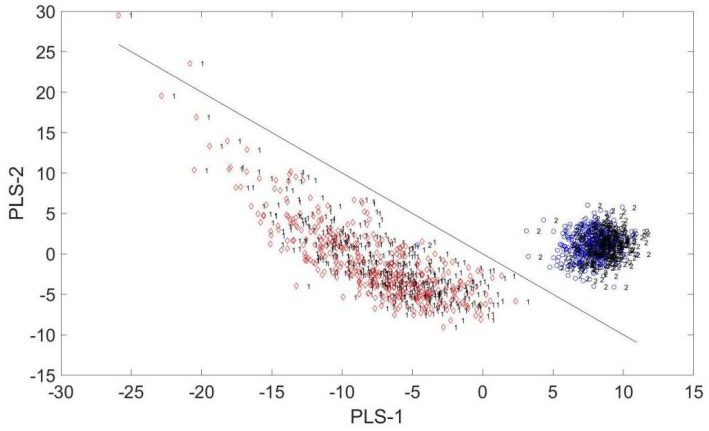


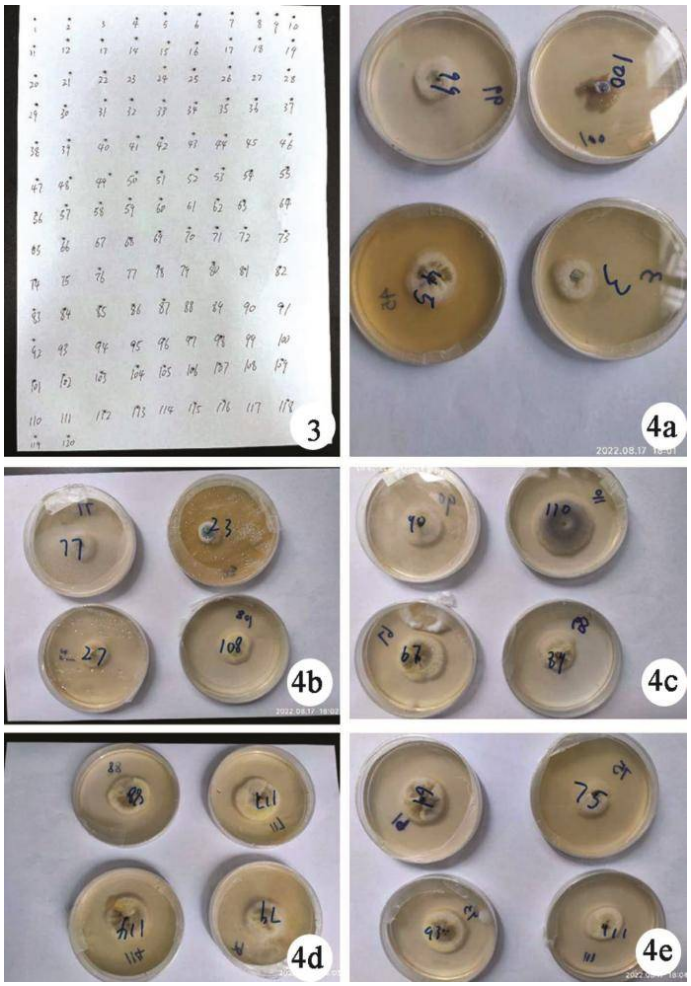
Fig. 2. Scatter plot of the PLS-DA model results.

Table 1. Classification accuracy of the PLS-DA model at different times after infection.

Length of time after infection (days)	Discrimination accuracy of the validation set (%)	RMSE (%)	Discrimination accuracy of the test set (%)	RMSE (%)	Overall discrimination accuracy (%)
3	100	0	100	0	100
5	94	6	98	2	96
7	97	3	97	3	97
9	96	4	96	4	96
11	97	3	97	3	97
13	99	1	99	1	99
15	96	4	97	3	96.5
17	97	3	97	3	97
19	95	5	97	3	96

The culture results showed that 19 seeds were infected by *P. decumbens*. Hence, the discrimination accuracy was 95% and the misidentification rate was 5%, which is acceptable for practical applications. The verification results are shown in Fig. 4. In summary, the on-line detection of *P. decumbens*–infected choy sum seeds can be realized based on near-infrared reflectance spectroscopy.

Based on the principle of near-infrared diffuse reflectance spectroscopy, this study analysed the near-infrared spectra of *P. decumbens*–infected choy sum seeds. The reflectance of the choy sum seeds infected with *P. decumbens* in the 950-1100-nm range was significantly different from that of the sterile choy sum seeds. The original near-infrared reflectance spectra of 400 sterile choy sum seeds and 400 *P. decumbens*–infected choy sum seeds were first preprocessed by SG convolution smoothing and then by SNV transformation. A prediction model for the qualitative identification of *P. decumbens*–infected choy sum seeds (the PLS-DA model) was constructed based on the preprocessed spectra, and the accuracy of the model was tested. Results showed that the discrimination accuracy of the PLS-DA model for *P. decumbens*–infected choy sum seeds was 99% for the calibration set and 99% for the validation set (root mean square errors both 1%). Hence, the overall discrimination accuracy was 99%. The discrimination accuracy of the PLS-DA



Figs 3-4. 3. Numbering of the 120 untreated choy sum seeds. 4a. *Penicillium decumbens*–infected choy sum seeds cultured on PAD plates. 4b. *Penicillium decumbens*–infected choy sum seeds cultured on PAD plates. 4c. *Penicillium decumbens*–infected choy sum seeds cultured on PAD plates. 4d. *Penicillium decumbens*–infected choy sum seeds cultu. 4e. *Penicillium decumbens*–infected choy sum seeds cultured on PAD plates.

model was verified to be 95% in 120 samples that were not used for modelling. The length of time after infection had little effect on the discrimination accuracy of the model. Thus, by near-infrared reflectance spectroscopy, seed-borne diseases in choy sum can be identified. With the innovative product developed here and the demonstration of its practical application, this work lays a foundation for the development of intelligent equipment to identify and filter out infected choy sum seeds and provides a decision-making basis for seed breeding industrialization and germplasm supervision.

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