Determination of soluble proteins and photosynthetic pigments of cherry tomato leaves using near-infrared spectroscopy

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Introduction

Cherry tomato is now a widespread type of table tomato in China. They are usually cultivated in soil-less, plastic greenhouses.[1] This cultivation needs precise water use and fertilizers. The content of soluble proteins and photosynthetic pigments are highly correlated with the nutrition condition of crops. Detection of these indices is mainly dependent on destructive methods. Near-infrared (NIR) spectroscopy is a rapid non-invasive method of analysis. Support vector machine (SVM) is a powerful method for performing nonlinear classification, multivariate function estimation or nonlinear regression, and has also led to many other recent developments in kernel-based learning methods in general. Least-squares support vector machines (LS-SVM), a reformulation of the SVM to avoid solving quadratic optimization process, was commonly used.[2] The objectives of this study are (1) to investigate the feasibility of using the NIR method to predict soluble proteins and photosynthetic pigments of cherry tomato leaves nondestructively; (2) to compare the performance of different chemometrics techniques, including preprocessing methods (SG, SNV, first and second derivative) and calibration models (BPNN, LS-SVM).

Materials and methods

Plant material

The experiments were conducted at the field research station of the Jiangsu University, in Zhenjiang, Jiangsu province, in China in greenhouses with polyethylene cover and without active climate-control systems. The two greenhouses had an east-west orientation, with crops rows aligned north-south. The cherry tomato crops were grown in 28 L styrofoam containers, measuring 37 cm × 27 cm × 28 cm deep, filled with perlite (3–6 mm diameter), which were placed

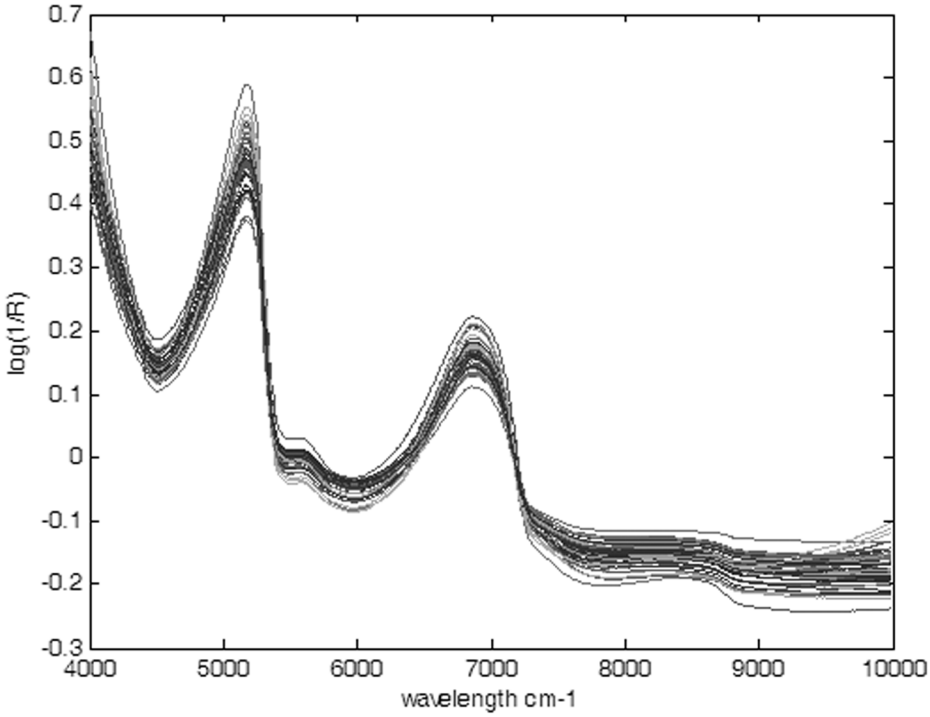

Figure 1. The NIR spectrum of cherry tomato leaves.

in gutters for the collection and re-circulation of drainage. Seedlings were grown in rockwool cubes and transplanted before the inflorescence of the first truss was visible. The 110 fresh leaves in different positions were separated and clarified as different samples.

Leaf soluble proteins and pigment quantification

The total soluble proteins were extracted by the method described by Laukkanen *et al*. (1997).[3] After pulverizing in liquid nitrogen, the leaf tissue was homogenized in 2 mL of extraction buffer (50 mmol L^{-1} Tris-HCl pH 8.6, 20 mmol L^{-1} KCl, 10 mmol L^{-1} MgCl2, and 1.5% (w/v) PVP), and the protein concentration was determined according to Bradford (1976)[4] after centrifuging the homogenate at 13,000g for 15min. The photosynthetic pigments in leaves were extracted in 80% acetone and centrifuged twice at 5000 g for 15 min. The concentrations of chlorophylls and carotenoids were determined spectrophotometrically and calculated per unit fresh mass basis using the equations of Lichtenthaler and Wellburn (1983).[5]

NIR spectra collection and Chemometrics analysis

The NIR spectra were collected in the reflectance mode using the Antaris II Near-infrared spectrophotometer (Thermo Electron Co., USA) with an integrating sphere. Each spectrum was the

average spectrum of 32 scans. The range of spectra was 10,000–4000 cm^{-1}, and the raw data were measured in 3.856 cm^{-1} intervals, which resulted in 1557 variables (Figure 1).

Partial least squares (PLS) analysis was the calibration method, with comparison of different spectral preprocessing by Savitzky-Golay (SG) smoothing, standard normal variate (SNV), first and second derivative. Simultaneously, certain latent variables (LVs) were used as the inputs of back-propagation neural network (BPNN) and least squares-support vector machine (LS-SVM) models. All calculations were made with a NIR spectra analysis toolbox developed in our Laboratory, and the program was implemented in the Matlab environment (The Math Works, Natick, USA).

Results and discussion

The descriptive statistics for soluble proteins, chlorophyll *a*, chlorophyll *b* and carotenoids of tomato leaves determined by standard laboratory methods are presented in Table 1.

The mean values of the soluble proteins, chlorophyll *a*, chlorophyll *b* and carotenoids were 0.121, 1.011, 0.458, 0.182 for all leaves samples with 60 in calibration set and another 50 in validation set. Some details of leaves parameters are summarized in Table 1.

In the raw spectra shown in Figure 1, a small offset can be observed at higher wave-numbers. The trend of spectra is very similar. Towards lower wave-numbers the spread of the spectra increases. PLS models were built after the spectra were preprocessed by SG smoothing, SNV, and first and second derivative. The best PLS models were obtained by first-derivative spectra for soluble proteins with 4 LVs, whereas the original spectra were used for chlorophyll *a* chlorophyll *b* and carotenoids, with 5, 5 and 6 LVs respectively. These LVs were used as the inputs of BPNN and LS-SVM models. LS-SVM with RBF was chosen to build models. The performance of BPNN and LS-SVM models are shown in Table1. All of the LS-SVM models outperformed PLS models and BPNN models. Figure 2 gives the plot of the predicted values by LS-SVM, the measured values of chlorophyll a in cherry tomato leaves.

The coefficients of determination (*R*), root mean square errors of prediction (*RMSEP*) and biases in the validation set by LS-SVM were 0.899, 0.084 and 0.1378 for soluble protein, 0.908,

Table 1. Range of reference values, near infrared (NIR) calibration and cross-validation statistics.

Compound	Range (mg g^{-1})	Mean (mg g^{-1})	Std	BPNN		LS-SVM	
				R	*RMSEP* (mg g^{-1})	*R*	*RMSEP* (mg g^{-1})
Chlorophyll *a*	0.493–1.832	1.011	0.817	0.885	0.0893	0.908	0.0615
Chlorophyll *b*	0.194–0.856	0.458	0.3935	0.891	0.0702	0.917	0.0514
Carotenoid	0.071–0.564	0.182	0.247	0.856	0.1231	0.896	0.0915
TSP	0.061–0.236	0.121	0.105	0.862	0.1021	0.899	0.084

TSP: total soluble proteins; Std: standard deviation; BPNN: Back-propagation neural network; LS-SVM: least squares-support vector machine; *RMSEP*: root mean square error of prediction; *R*: correlation coefficient.

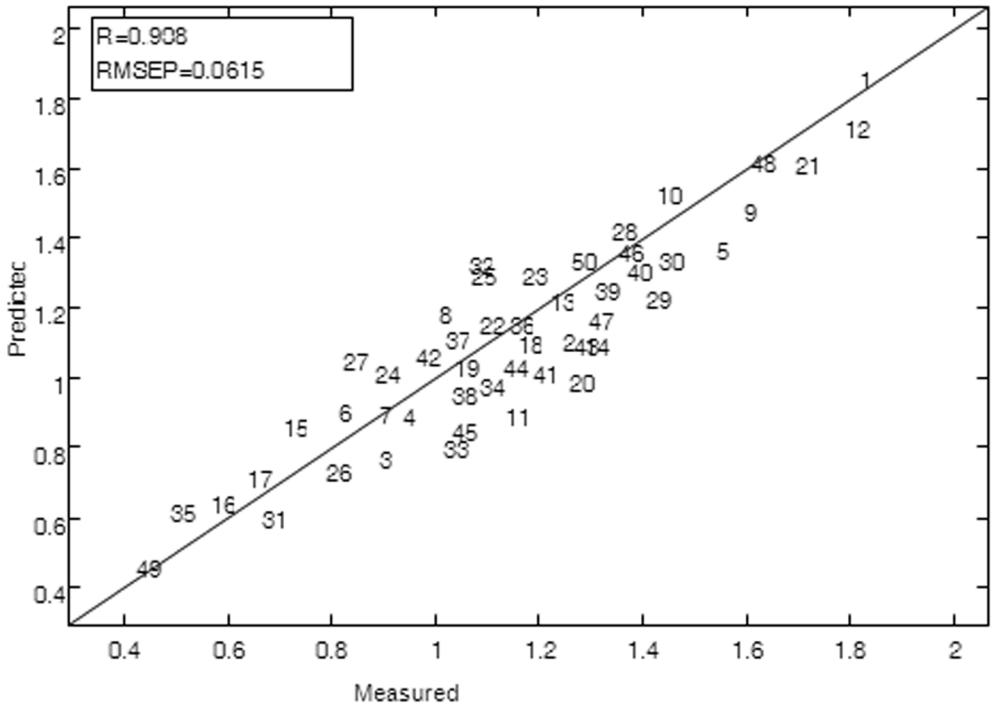

Figure 2. The plot of predicted values by LS-SVM and measured values of chlorophyll a in cherry tomato leaves.

0.0615 and 0.099 for chlorophyll *a*, 0.917, 0.0514 and 0.079 for chlorophyll *b*, and 0.896, 0.0915 and 0.239 for carotenoids, respectively.

Conclusion

The results indicated that NIR spectroscopy combined with PLS and LS-SVM could be successfully applied for the determination of photosynthetic pigments, and for the estimation of carotenoids and protein content of cherry tomato leaves. The results would be helpful for further in-field analysis, using NIR spectroscopy to monitor the growing status and physiological properties of cherry tomatoes. Further studies are proposed to apply the NIR method to study changes in soluble proteins and photosynthetic pigments of cherry tomato leaves, under the influence of various fertilizers.

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