Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2015, 22(5): 245−249

Discrimination of Transgenic Rice Based on Near Infrared Reflectance Spectroscopy and Partial Least Squares Regression Discriminant Analysis

ZHANG Long[1, 3], WANG Shan-shan[2], DING Yan-fei[2], PAN Jia-rong[2], ZHU Cheng[2, 3]

([1]*College of Ecology, Lishui University, Lishui 323000, China;* [2]*College of Life Sciences, China Jiliang University, Hangzhou 310018, China;* [3]*College of Life Sciences, Zhejiang University, Hangzhou 310058, China*)

Abstract: Near infrared reflectance spectroscopy (NIRS), a non-destructive measurement technique, was combined with partial least squares regression discrimiant analysis (PLS-DA) to discriminate the transgenic (TCTP and mi166) and wild type (Zhonghua 11) rice. Furthermore, rice lines transformed with protein gene (*OsTCTP*) and regulation gene (*Osmi166*) were also discriminated by the NIRS method. The performances of PLS-DA in spectral ranges of 4 000–8 000 cm^{-1} and 4 000–10 000 cm^{-1} were compared to obtain the optimal spectral range. As a result, the transgenic and wild type rice were distinguished from each other in the range of 4 000–10 000 cm^{-1}, and the correct classification rate was 100.0% in the validation test. The transgenic rice TCTP and mi166 were also distinguished from each other in the range of 4 000–10 000 cm^{-1}, and the correct classification rate was also 100.0%. In conclusion, NIRS combined with PLS-DA can be used for the discrimination of transgenic rice.

Key words: near infrared reflectance spectroscopy; genetically-modified food; regulation gene; protein gene; partial least squares regression discrimiant analysis

Genetically-modified foods (GMOs) are inserted with foreign genes which increase resistance to diseases, pests and herbicides or improve nutritional contents which never occur naturally (Anklam et al, 2002). Since it is suspected with the safety about environmental hazards, human health risks, and economic concerns, the public are scared of consuming GMOs, and the GMOs are severely rejected in many regions of the world. In order to regulate the introduction and production of GMOs, many regulations and legislations are promulgated with the Ministry of Health, the Ministry of Agriculture, the General Administration of Quality Supervision, and the Inspection and Quarantine in China. Thus, the fast reliable detection methods must be developed to support the regulations and legislations above.

There are many detection methods for transgenic food. Western blot (Lipton et al, 2000; Sambrook and Russel, 2000), enzyme linked immunosorbent assays (Yates, 1999; Margarit et al, 2006) and lateral flow strip (Fagan et al, 2001) are protein-based methods. Southern blot (Ross et al, 1999; Stull, 2001), qualitative polymerase chain reaction (qPCR) (van Hoef et al, 1998; Lipp et al, 1999; Singh et al, 2007), microarray (Miraglia et al, 2004) and real time PCR (Heid et al, 1996; Ahmed, 2000; Mäde et al, 2006; Akiyama et al, 2007; Grohmann and Mäde, 2009) are DNA-based methods. Additionally, high performance liquid chromatography and gas chromatography are also useful technologies (Alishahi et al, 2010). However, these technologies had many shortcomings, such as high cost, difficult to use, special need, and long duration.

Near infrared reflectance spectroscopy (NIRS) is sensitive to organic compounds with vibration overtones of C–H, O–H and N–H, which is rapid, lower cost and non-destructive. The content of modified DNA is in amount of ultra trace, so detection of the modified DNA is difficult to identify the GMOs, while detection of the products resulting from the genetic modification (particular proteins) or larger structural changes (phenotype) are feasible (Munck et al, 2001; van Duijn et al, 2002; Xie et al, 2007a, b; Alishahi et al, 2010). In previous studies, NIRS has successfully used in detection of

Received: 11 November 2014; **Accepted:** 26 May 2015
Corresponding author: ZHU Cheng (pzhch@zju.edu.cn)

Peer review under responsibility of China National Rice Research Institute
http://dx.doi.org/10.1016/j.rsci.2015.09.004

transgenic tomato (Xie et al, 2007a), barley (Hurburgh et al, 2000) and corn (Rossel et al, 2001) or mutant of barley (Munck et al, 2004) and corn (Campbell et al, 2000).

Rice is relatively easy to consult transgenic. There are rarely studies conducted on the detection of transgenic rice to our knowledge (Jiao et al, 2010). Translationally-controlled tumor protein (TCTP) is most likely controlled by a cell housekeeping gene which presents as a multifunctional protein and essential in the development of mammals, higher plants and *Saccharomyces cerevisiae*. It was recently discovered that *OsTCTP* works on mercury resistance. As we all known, microRNAs (miRNAs) are a class of small non-coding RNAs which negatively regulate specific target mRNAs at the post-transcriptional level. Thus, cd-responsive gene *Osmi166* were selected (Ding et al, 2011).

In the present study, heavy metal responsive genes *OsTCTP* and *Osmi166* transgenic rice were employed to discriminate the transgenic rice (Wang, 2010; Ding et al, 2011). The aim of the present study was to discriminate the transgenic and wild type rice, and the transgenic rice transformed with protein gene and regulation gene. Additionally, the partial least squares regression discrimiant analysis (PLS-DA) was employed as a discriminant model.

MATERIALS AND METHODS

Transgenic rice

Wild type rice Zhonghua 11 (*Oryza sativa* L. subsp. *japonica*, ZH11) was used as the transgenic material. Two single-copy transgenic rice lines were developed in the authors' laboratory by respectively introducing *OsTCTP* and *Osmi166* genes into ZH11 using an *Agrobacterium*-mediated transformation method. The rice seed used were all T_3 homozygous lines and designated as TCTP and mi166, respectively. *OsTCTP* and *Osmi166* genes expressed in rice plants and the expression levels were stable. The rice lines were all planted and harvested under natural condition and dried in the sun in the same field.

NIRS analysis

The near infrared reflectance spectra of rice grains were scanned on a Nicolet Nexus 870 FT-IR spectrometer (Thermo Corporation, USA) with the mode of diffuse transmission. The spectra of 192 rice grain samples were measured at 8 cm^{-1} resolution with 32 scans above the detection window. And both the obverse and the reverse of the grains (three grains for each sample) were scanned. The spectra were collected with the OMNIC 6.0 software in range of 4 000–10 000 cm^{-1} in the absorbance mode. Each spectrum was structured in 3040 data with the format of ASCII which are easily combined with the Matlab software version 6.5.

PLS-DA

PLS is a well-established multivariate regression model which constructs a mathematic relationship between descriptors and dependent variables (Kleinbaum et al, 1988). In the PLS model, principal component analysis (PCA) was first processed, and then the scores of principal components (PCs) obtained were considered as new eigenvectors of the original spectra. The performance of PLS model is affected by the number of PCs (Marengo et al, 2008). In order to discriminate the transgenic and wild type rice, PLS was used as discrimination by designed the values of different category. The values of transgenic (TCTP and mi166) and wild type (ZH11) rice were designated as '-1' and '1', respectively, in the PLS-DA model. To identify rice transformed with protein gene and regulation gene, TCTP and mi166 were used and the values were designated as '-1' and '1' in the PLS-DA model, respectively. The predicted values below '0' were considered to the category of '-1', and the values above '0' were considered to the category of '1'.

Leave-one-out cross-validation was used for the calibration, which involved in using a single observation from the original sample as the validation data and the remaining observations as the training data.

Model evaluation

The PLS-DA model was evaluated with the parameters of root-mean-square error (RMSE) and correlation coefficient (r^2). RMSE used for calibration was designated as root mean square error in calibration (RMSEC) and for validation or prediction as root mean square error in prediction (RMSEV). r^2 used for calibration was designated as r^2_c and for validation as r^2_v.

RESULTS AND DISCUSSION

Diffuse transmittance spectra of rice grains

Spectra of transgenic rice and wild types ZH11 in the region of 4 000–10 000 cm^{-1} are shown in Fig. 1. It is obviously that the shapes of the spectra of TCTP, mi166 and ZH11 were overlapped and quite homogeneous and cannot be identified by naked eyes, but there were some variants in the 4 000–10 000

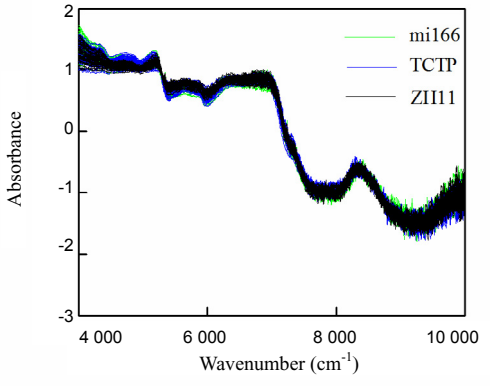

Fig. 1. Standard normal variance pretreated spectra of rice grains.
ZH11, Zhonghua 11.