Near infrared spectroscopic study of tobacco plants engineered with potato virus Y CP DNA

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Introduction

The increasing number of genetically modified organisms (GMO) challenged development of new approaches for effective screening, identification and conformation of presence / absents of genetic modifications. The qualitative (classification) analysis requires the development of a model which defines the mean and standard deviation of each sample type in multidimensional space followed by the testing to which group an unknown sample belongs. Near infrared (NIR) spectroscopy provide a total chemical profile of a sample and may strengthen the classification power for further data analysis. However, only a few studies for NIR detection of GMO have been reported. Recently, NIR spectroscopy has been used in attempts to distinguish Roundup Ready Soybeans (RRS) from conventional soybean.[1] In this study, transmittance spectral scans were taken from three Infratec 1220 spectrometers where whole-grain samples flow through a fixed path length. Locally weighed regression using a database of approximately 8000 samples was 93% accurate for distinguishing RRS from unmodified soy.

In the present study the NIR transflectance spectroscopy was employed for classification of transformed and non-transformed tobacco plants with coat protein (CP) of potato virus Y (PVY).

Materials and methods

The transgene construct carrying the whole CP gene of PVY, supplemented with the translational start signals was prepared by van der Vlug *et al*.[2]

Tobacco leaf disks from in vitro plants were transformed by the *Agrobacterium tumefaciens* C58 strain LB4404 containing the pTCPY2 plasmid.

The tobacco leave samples (18 non-transformed and 17 transformed) were collected from plants, grown up in cultivation room under controlled light, temperature, humidity and soil conditions. The tobacco cultivar was Bulgarian oriental type—Rila $_{89}$.

The NIR transflectance spectra (1100–2500 nm) were collected with Foss NIRSystem, model 6500 in sample cup cell with golden reflector.

The discriminant analysis of the NIR spectra was performed with the SPSS 8.0 software.

Results and discussion

The transflectance NIR spectra were read from fresh, ground whit silica gel and lyophilised leave samples. The raw NIR spectra of transformed and wild genotype tobacco leaves for all of the used sample preparations did not show marked differences (Figure 1).

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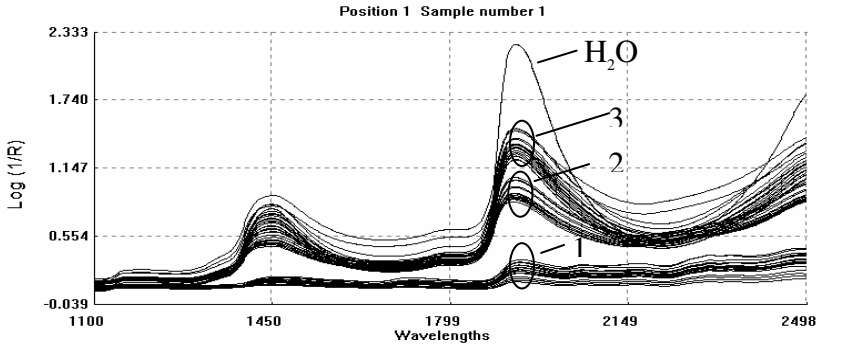

Figure 1. NIR transflectance spectra of: 1, lyophilized tobacco leaves; 2, ground with silica gel and 3. fresh tobacco leaves.

The NIR spectra of fresh tobacco leaves were very similar to that of water. The spectral features were with enhanced differentiation by utilizing second-derivative algorithm. The linear regression model was employed for wavelength selection and the classification rule was developed by carrying out the discriminant analysis. A proper classification of studied genotypes was achieved by eight factor discriminant model only with NIR transflectance spectra of fresh tobacco leaves. The trasngene and wild genotype was correctly classified whit 91,5% accuracy (estimated by cross validation, Table 1). The miss-classification of ground with silica gel and lyophilised tobacco leave samples was greater than 30%. Because the sample preparations affect only hydrogen-bonding environment and content of water respectively and did not exchange the chemical composition of the sample, the correct classification of the gene-modified plants appear to be due to detected structure of water continuum in fresh tobacco leaf. Moreover in transgenic plants CP specific RNA transcript were produced, but accumulation of viral CP was not detected (by several sensitive immunological techniques:[3] double antibody sandwich ELISA and Western blot analysis of total immunoprecipitated total leaf protein), which mean that the variation of biological information on the RNA sequence level is also represented at the phenotypic level read by the NIR transflectance spectroscopy.

Table 1 Classification results.[b,c]

			Predicted Group Membership		Total
			Rila 89	*Transgene line 224*	
Original	Count	*Rila 89*	17	1	18
		Transgene line 224	1	16	17
	%	*Rila 89*	94.4	5.6	100.0
		Transgene line 224	5.9	94.1	100.0
Cross-validated[a]	Count	*Rila 89*	16	2	18
		Transgene line 224	1	16	17
	%	*Rila 89*	88.9	11,1	100.0
		Transgene line 224	5.9	94.1	100.0

[a]Cross-validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

[b]94,3% of original grouped cases correctly classified.

[c]91,5% of cross-validated grouped cases correctly classified

Conclusion

The comparative study of NIR transflectance spectra of fresh, ground with silica gel and lyophilized tobacco leaves could be assumed as evidence that the water structured in a biological matrix made the differences on DNA level detectable for NIR.

References

1. S.A. Roussel, C.L. Hardy, C.R. Hurburgh, Jr, and G.R. Rippke, *Appl. Spectrosc*. **55(10),** 1023 (2001).
2. R. van der Vlugt, R. Ruiter and R. Godbach, *Pl. Mol. Biol*. **20,** 631 (1992).
3. I. Kamenova, M. Prins, D. Peters, New aspects of resistance research on cultivated plants: Proceedings of the 7th Ascherleben symposium, Ed. by W. Flamme and G. Lusewitz, Heft 3, Germany p. 115 (2000).