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Discrimination between Nongenetically Modified (Non-GM) and GM Plant Tissue Expressing Cysteine-Rich Polypeptide Using FT-Raman Spectroscopy

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Fourier transform (FT)-Raman spectroscopy was applied to the analysis of genetically modified (GM) plant tissue. Transgenic carrot callus and tobacco plants possessing a novel *StSn1* gene coding for a cysteine-rich snakin-1 polypeptide were obtained after *Agrobacterium*-mediated transformation. The presence of the *StSn1* gene and its expression were confirmed by polymerase chain reactions using plant DNA and cDNA as templates for the amplification of the transgenes. Raman measurements were taken from lyophilized GM carrot callus tissue, fresh GM tobacco leaves, and from seeds produced by GM tobacco plants as well as from the nontransformed controls. Cluster analysis applied to the obtained spectra allowed clear separation of the GM samples expressing the *StSn1* gene and the nontransformed control to distinct groups. Such discrimination was achieved only when wavenumber ranges around 500 cm^{-1} were analyzed. The results indicate that discrimination between the GM and non-GM materials was related to S−S stretching vibrations in snakin-1, as it contained six sulfur bridges. Other introduced genes, neomycine phosphotransferase (*nptII*) and Chitinase (*chit36*), did not cause any detectable changes by Raman spectroscopy in plant tissue. This is the first report on the use of Raman spectroscopy for a nondestructive analysis of GM plant material expressing the gene coding for a cysteine-rich polypeptide.

KEYWORDS: Carrot; *Daucus carota*; FT-Raman spectroscopy; genetically modified (GM) plants; plant transformation; *Nicotiana tabacum*; snakin-1; tobacco

INTRODUCTION

The development of genetic transformation techniques enables nowadays a transfer of genes between unrelated organisms. Transgenic science and technology have expanded rapidly in the recent years resulting in the development of novel genetically modified (GM) crops, which are currently grown on over 100 million ha worldwide (7% of total world production area) by more than 10 million farmers (*1*). However, the impact of GM organisms (GMO) on human health and on the environment is still a principal safety issue addressed, which restricts implementation of GM technology into agricultural practice in many countries (*2*).

The most common technique for transformation of higher plants utilizes *Agrobacterium tumefaciens* as a vector. This bacterium has the ability to transfer T-DNA, a fragment of plasmid DNA, into plant cells where it is integrated into the plant genome. In consequence, the genes delivered to the host cells, the transgenes, can be functionally expressed, and new proteins can be synthesized by the host (*3*). Prior to the transformation process, a plasmid T-DNA can be engineered to contain a gene of interest coding for a specific protein and a selection or reporter gene, which allows the selection of successfully transformed cells (*4*). One of the most commonly used selection genes is *nptII* coding for neomycin phosphotransferase conferring resistance to the antibiotic kanamycin. Successful delivery of the T-DNA to the host, its integration with the genome, and then functional expression of the transgenes can be monitored by screening the host cells or tissue challenged to kanamycin (*5*). The selected GM cells or tissues can then be directed to regenerate whole plants. The obtained material is verified for the presence and expression of the delivered gene of interest using molecular techniques. They usually rely on the amplification of the inserted DNA fragment using polymerase chain reaction (PCR). Moreover, the technique is useful for the analysis of messenger RNA (mRNA), which allows confirmation whether the transgene can be functionally expressed to the protein (*6*). PCR methods are also suitable for

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the identification of GMO and products containing GMO. Thus, they are implemented into standard procedures in authorized reference laboratories for the detection of possible contamination of conventional products with GMO to ensure safety regulations. Although techniques of nucleic acid analysis provide reliable information, they are expensive and require DNA extraction from the sample, so the biological material is destroyed (*7*). Thus, there is a great need for new methods that could be useful in the analyses of living GM material, and which would contribute to further understanding and control of plant transformation technology.

During the past decade, analytical capabilities of Raman spectroscopy have increased considerably, mainly because of the technical improvements of the instrumentation and the development of chemometric algorithms useful for data analysis. Nowadays, Raman spectroscopy can be successfully applied for the extraction of information from complex multivariate systems, such as plants. Living plant tissues contain enzymes and coenzymes that absorb in the visible spectral range, and so, they may be destroyed by photochemical reactions. However, excitation in the near-infrared range (NIR) is not absorbed by most samples; thus, plant tissue does not excite fluorescence, and its thermal breakdown can be reduced to a minimum. Additionally, by using a Michelson interferometer and Fourier transform (FT) processors for analysis of scattered light, an enhancement of the recorded intensity is observed (*8*). First applications of NIR-FT-Raman spectroscopy for nondestructive measurements of various plant tissues were found to be very promising, and this method has been therefore used extensively for this purpose in the past few years (*9*). Samples can be analyzed at ambient temperature and pressure without any need for preprocessing. The main advantage of NIR-FT-Raman spectroscopy of fresh plant material is that water due to its low polarizability has only low response, in contrast to more commonly applied infrared (IR) spectroscopy. Therefore, both dried and fresh samples can be used successfully for Raman measurements (*10*).

Raman spectroscopy can be successfully applied for identification of various plant components if characteristic key bands of the individual analyte molecules are found in the spectrum. Based on such marker bands, spectroscopic analyses allow discrimination between different plant species and even chemotypes of the same species (*11*). When a visual inspection of the spectra does not allow confident assignment of a band to the analyte, chemometric approaches may provide useful information, particularly when several minor bands dispersed throughout the spectrum must be considered simultaneously (*12*).

In the present work, we applied FT-Raman spectroscopy for the analysis of various genetically modified (GM) plant tissues to evaluate its usefulness for the detection of genetic changes. For this purpose, we developed transgenic carrot and tobacco with introduced *StSn1* gene coding for snakin-1 polypeptide. Snakin-1 was originally found in potato; it is rich in sulfur present in 12 cysteine groups, and it exhibits antimicrobial activity (*13*). The results obtained from our Raman measurements of carrot and tobacco tissues as well as tobacco seeds indicate that Raman spectroscopy can be considered as a potential analytical technique allowing the discrimination of conventional plant material from their GM analogues expressing cysteine-rich polypeptide.

MATERIALS AND METHODS

Genetic Transformation. Genetic transformation was performed using *Agrobacterium tumefaciens* strain EHA105. The bacterial cells possessed binary plasmids either pGJ1165 or pGJ2350, which were developed and provided by Dr. G. Jach (Max Planck Institute for Plant Breeding Research, Cologne, Germany). The T-DNA of pGJ1165 plasmid carried snakin-1 (*StSn1*) gene (GenBank No. AF014396), and the T-DNA of pGJ2350 carried the sequence of CHIT36 endochitinase (*13, 14*). Both genes were flanked by the CaMV 35S promoter and terminator for constitutive expression. Additionally, the T-DNA of both plasmids contained the neomycin phosphotransferase (*nptII*) selection gene conferring kanamycin resistance. Transformation of carrot (*Daucus carota*) cv. 'Dolanka' was done by cocultivation of suspension cell culture with *A. tumefaciens* cells containing pGJ1165 plasmid, and the putatively transformed cells were maintained for the growth of unorganized callus tissue according to the protocols described previously (*15*). Leaf discs of aseptic tobacco (*Nicotiana tabacum*) plants cv. 'Samsun' grown in vitro were submerged for 10 min in liquid Murashige and Skoog (*16*) medium with 20 g/L sucrose (MS20) and 100 μl inoculum ($OD_{600} = 0.5$) of *A. tumefaciens* cells containing either pGJ1165 or pGJ2350 plasmid in MS medium. The discs were placed on 7% agar solidified MS20 with 1 mg/L 6-benzylaminopurine in Petri dishes and incubated for 48 h at 25 °C in the dark. Then, they were transferred to the same medium supplemented with antibiotics, 100 mg/L kanamycin for the selection of the transformants and with 400 mg/L cefotaksime to kill the bacteria, and incubated at 25 °C in a 16 h photoperiod. Developing shoots were further cultured on MS20 medium with antibiotics for shoot growth and rooting. The rooted plantlets were transferred to pots with peat in a climate chamber (22/19 °C day/night, 16 h photoperiod) where they grew until the seeds were produced after self-pollination. The nontransformed control carrot callus and tobacco plants were obtained in analogous way, except no bacterial cells were added into the inoculum and there was no antibiotic selection applied.

Molecular Analyses of Transgenics. The presence of the *StSn1* gene in the obtained material was confirmed by the isolation of plant DNA and the amplification of the DNA fragment using the polymerase chain reaction (PCR) technique in a Master Cycler thermocycler (Eppendorf, Germany) and recombinant *Taq* polymerase (Fermentas, Germany). The reactions were performed according to the protocol described previously (*17*) using 60 °C annealing temperature and specific primers to detect sequences of *StSn1* (forward: 5′ CCACGTCTTCAAAGCAAGTGG 3′ and reverse: 5′ ATTGTGTGTGGCTGTCAAAA 3′) and *nptII* (forward: 5′ CGCAGGTTCTCCGGCCGCTTGGGTGG 3′ and reverse: 5′ AACTCGTCAAGAAGGCGATAGAAG 3′).

The transgene expression and the presence of snakin-1 transcript in plant tissue were verified using a reverse transcribed (RT)-PCR. For this purpose, RNA was extracted using a commercial RNeasy Plant Mini Kit (Qiagen, Germany), DNA in RNA sample was digested with deoxyribonuclease I (Fermentas, Germany), and the cDNA was synthesized from the obtained RNA using the First Strand cDNA Synthesis Kit (Fermentas, Germany) according to the manufacturer protocol. PCR with the specific primers was done as described above by using the obtained cDNA as the template. Additionally, a pair of ipicar-F and ipicar-R4 primers specific to a constitutive isopentenyl pyrophosphate isomerase (*ipi*) gene was used as the control of RNA isolation and cDNA transcription (*15*).

Seed Germination Test. Tobacco seeds collected from the nontransgenic control and transgenic plants were surface sterilized in 70% ethanol for 5 min, washed in sterile water, and placed on MS20 medium with 100 mg/L kanamycin. The seeds were germinated at 25 °C in a climate chamber (22/19 °C day/night, 16 h photoperiod). After 5 weeks, the number of seedlings that survived and died was recorded, and the segregation ratio of kanamycin resistant to sensitive seedlings was used to test the hypothesis of independent gene insertions using the χ^2 test.

Raman Measurements. Raman spectra were recorded using a FT-Raman Spectrometer Nicolet NXR 9650 appointed with a Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. The instrument was equipped with x-y-z automatic 'MicroStage', a mirror objective, and a prism slide for redirection of the laser beam. Compared with the standard vertical sampling arrangement, the samples were mounted horizontally.

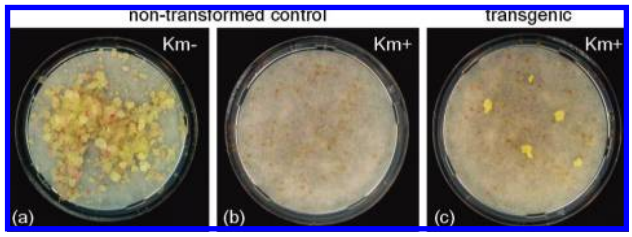

Figure 1. Carrot callus tissue from the nontransformed control cells (**a**) developing on medium devoid of kanamycin and (**b**) inhibited cell development on the selection medium with kanamycin. (**c**) Individual calli developing from carrot cells after *Agrobacterium*-mediated transformation with pGJ1165 plasmid containing *nptII* gene conferring resistance to kanamycin on medium enriched with this antibiotic.

Spectral measurements were taken from genetically modified and the nontransformed control of lyophilized carrot callus, fresh tobacco leaves, and tobacco seeds. All spectra were performed with a spectral resolution of 4 cm^{-1} in the range from 100 to 4000 cm^{-1} with an unfocused laser beam of 150 mW of a diameter about 100 μm. Measurements of carrot callus and tobacco seeds were obtained with 128 scans, whereas from tobacco leaves 256 scans were taken.

Raman spectra were registered by the Omnic/Thermo Scientific software. Hierarchical cluster analysis was performed for the obtained spectra by applying the Opus/Bruker package. The spectra were not baseline corrected. Individual spectral distances were calculated with the standard or factorization algorithms after applying vector normalization. The cluster analysis was performed separately for each sample type for the whole as well as for specific wavenumber ranges using Ward's algorithm, as described later in the Results and Discussion.

RESULTS AND DISCUSSION

Development and Molecular Characterization of Transgenics. *Agrobacterium tumefaciens* is commonly used for genetic transformation of higher plants including carrot and tobacco (*18*). Its ability to transfer plasmid T-DNA allows the introduction of foreign genes into the plant cell genome and their subsequent expression. In this study, the pGJ1165 plasmid T-DNA contained two genes fused to the regulatory elements, the *StSn1* coding sequence of a cysteine-rich snakin-1 polypeptide and the *nptII* gene conferring resistance to kanamycin. The presence of the *nptII* gene in the same construct allowed growth and development of putatively transformed cells in the presence of kanamycin, which is a standard procedure used for the selection of transgenic tissue (*5*). Utilizing this approach, we observed that, after *Agrobacterium*-mediated transformation, some carrot cells could survive on the selection medium and developed into callus pieces, which were considered as putatively transformed (**Figure 1**). As the callus pieces developed from independent transformation events, they were analyzed individually. In contrast, the nontransformed control cells died soon after the exposure to antibiotic; thus, no callus developed. A similar reaction was observed for the control tobacco leaf discs, which turned white and died on the selection medium. The discs inoculated with *Agrobacterium* partially turned white but also retained green areas, particularly at the disc edge, which were exposed to *Agrobacterium* infection during the transformation process. Putatively transgenic green shoots developing from these sites and showing typical morphology were transferred to new media for the whole plant regeneration and subsequent seed production.

Carrot callus and tobacco plants selected on the kanamycin enriched medium were verified for the presence of the transgenes. PCR analyses were planned to amplify the sequences of the *StSn1* and *nptII* genes. The amplified DNA fragments

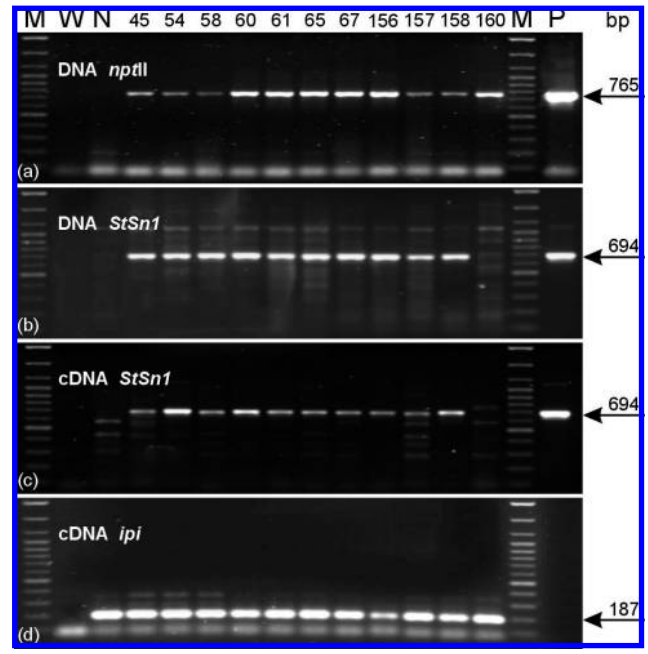

Figure 2. Products of DNA amplification after polymerase chain reaction (PCR) using (**a**, **b**) DNA or (**c**, **d**) cDNA from carrot callus as the template and using primers specific to neomycin phosphotransferase (*nptII*), snakin-1 (*StSn1*), and isopentenyl pyrophosphate isomerase (*ipi*) genes. Lanes: M = 100 bp DNA molecular marker; W = water; N = nontransformed control; P = pGJ1165 plasmid DNA; 45−160 = independent transgenic callus.

separated in agarose gels were the same size as those obtained from the amplification using a pure plasmid DNA template control and as the expected sizes deduced from the known DNA sequence. Thus, for the *StSn1* and *nptII* genes, the 694 bp and 765 bp fragments were detected, respectively (**Figure 2**). The presence of the transgene sequences in plant DNA confirmed transgenic character of the tissues. No amplification of the expected fragments was obtained for the nontransformed control. Only those accessions, which possessed both genes and the controls, were used for further molecular analyses and Raman measurements. Moreover, callus no. 160 containing the *nptII* gene but lacking the *StSn1* gene was used as an additional control.

A reverse transcriptase was used to synthesize cDNA from RNA, and then, PCR with specific primers was applied to confirm that the *StSn1* gene was functioning and could be expressed in plant tissue. Analogous to PCR with DNA, the reactions with cDNA used as a template resulted in the amplification of the product of the expected size (694 bp) for both the carrot and tobacco samples (**Figure 2**). Proper RNA isolation and cDNA synthesis was confirmed by the amplification of a housekeeping *ipi* gene using the cDNA template. The expected product of 187 bp was found in all plant samples, including the nontransformed control and callus no. 160. The results of molecular analyses indicate that GM material contained the *nptII* and *StSn1* functional genes, except carrot callus no. 160, which passed through the transformation procedures but did not contain the *StSn1* gene, thus in a consequence, could not express snakin-1 polypeptide.

Transformation of tobacco was done using two plasmids differing in their T-DNA content. Both plasmids contained the *nptII* gene but the pGJ2350 plasmid possessed, instead of the *StSn1* gene, the *chit36* gene coding for an endochitinase enzyme. Therefore, two types of transgenic tobacco were produced, the plants containing and expressing *StSn1* gene (plants nos. 109a

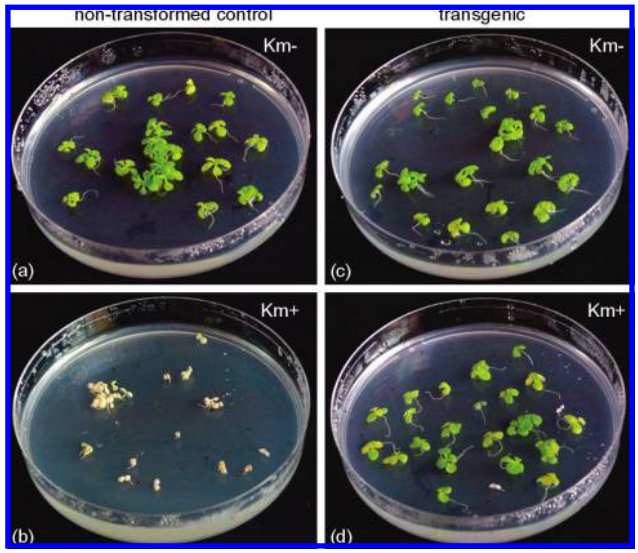

Figure 3. Tobacco seedlings from the nontransformed control seeds (**a**) developing on medium devoid of kanamycin and (**b**) died after the exposure to kanamycin. (**c**, **d**) Seedlings from seeds of transgenic plant 109A after *Agrobacterium*-mediated transformation with pGJ1165 plasmid containing the *nptII* gene conferring resistance to kanamycin, surviving (green) and sensitive (white) to kanamycin seedlings growing on medium (**c**) devoid of antibiotic and (**d**) supplemented with kanamycin.

Table 1. Resistance of Tobacco Seedlings to Kanamycin

plant no.	no. of seedlings: total	no. of seedlings: kanamycin resistant	%	expected segregation for 2 independent gene insertions	χ^2	p
nontransformed control	136	0	0			
108a	243	224	92	15:1	1.01	0.314
108b	286	272	95	15:1	0.75	0.388
109a	230	218	95	15:1	0.42	0.518
109b	347	324	93	15:1	0.08	0.773

and 109b) and the transgenic plants lacking this gene (plants nos. 108a and 108b); however, both transgenic tobacco contained *nptII* gene conferring kanamycin resistance. Therefore, transgenic plants nos. 108a and 108b did not synthesize snakin-1 and could be used for comparison in Raman measurements, in addition to the nontransformed control.

Despite molecular confirmation of the transgene integration and expression in tobacco plants, tobacco seeds produced by the obtained plants were challenged to kanamycin treatment. The seeds of the nontransformed control germinated, but the seedling growth was restricted, and all seedlings turned white and died during a four week incubation while the seedlings on the antibiotic free medium remained green and continued their growth (**Figure 3**). Most seeds collected from the transgenic tobacco plants germinated also in the presence of kanamycin and developed into normal and green seedlings (**Table 1**). Only a small fraction of seedlings were kanamycin sensitive (5−8%) and died. These results confirmed the transfer of the transgene from the parental T_0 plants to the next progeny and its functional expression in the developing seedlings. The occurrence of resistant and sensitive seedlings in one seed lot resulted from transgene segregation in the T_1 progeny (**Table 1**), which is in agreement with the expected segregation ratio for two independent insertions of the transgene in the T_0 plants (*19*).

Raman Discrimination between GM and Non-GM Tissue. Fourier transform infrared (FT-IR) spectroscopy has been recently successfully utilized for the assessment of tissue metabolomic fingerprints. Cluster analysis applied to IR spectra of freeze-dried plant samples allowed the discrimination of various plant species, populations, single plants, as well as the detection of genetic rearrangements related to chromosomal substitutions (*20*). In the present work, FT-Raman spectroscopy, with high excitation wavelength and low energy, has been considered as a potential analytical tool for discrimination between conventional plant materials and their GM isogenics expressing a novel polypeptide. Several Raman vibrational modes are useful for the interpretation of various amino acids and proteins occurring in plant tissue (*10*). Three signals coming from the CONH group are commonly used for the identification of different protein backbone conformations: amide I detected between 1680 and 1600 cm^{-1} (stretching vibration of C═O), amide II observed in the range between 1580 and 1480 cm^{-1}, and amide III between 1300 and 1230 cm^{-1} (amide II and III are associated with coupled C−N stretching and N−H bending vibrations of the peptide group) (*21*). Raman vibrational modes of amide groups are excellent markers of different proteins and their conformations when the measurements are performed from isolated or concentrated compounds. However, the investigation of living plant tissue is difficult because of the presence of hundreds of different proteins and several other plant components; for example, in the range of the amide I band, lignin and lipid signals are also pronounced. Thus, in the present work, single Raman spectra obtained from the GM and non-GM carrot and tobacco samples did not show any distinctive differences, which could be used for the analytical purpose.

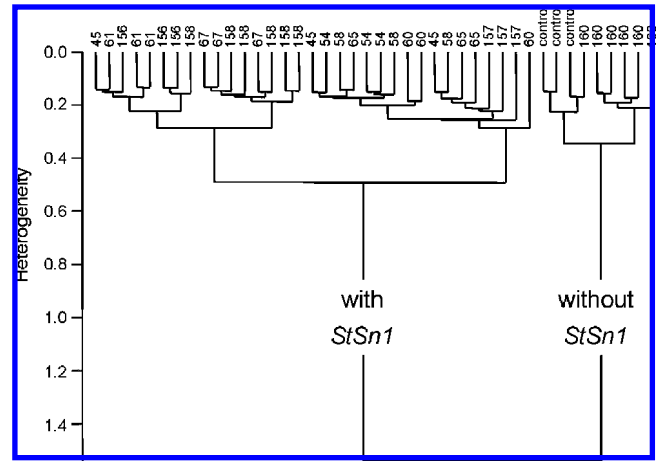

Figure 4. Dendrogram showing classification of carrot callus tissues into two distinct groups after cluster analysis of the single spectrum at the wavenumber range 620−500 cm^{-1} using the standard algorithm for calculation of spectral distances and the Ward's algorithm. At least three measurements were taken from each sample. Control = the nontransformed callus; individual numbers = independent genetically modified callus tissues; *StSn1* = snakin-1 gene.

To find meaningful and systematic differences among the measured spectra of the GM and non-GM material, cluster analysis was applied. Despite application of the standard procedures of the spectra pretreatment as well as various mathematical algorithms no discrimination could be achieved if the whole spectral range was considered. Analyses based on various narrow wavenumber ranges gave similar outputs except when a range between 620 and 500 cm^{-1} was applied (**Figures 4–6**). These results indicate that changes of plant constituents caused by genetic transformation were detectable by using Raman spectroscopy only in this specific wavenumber range.

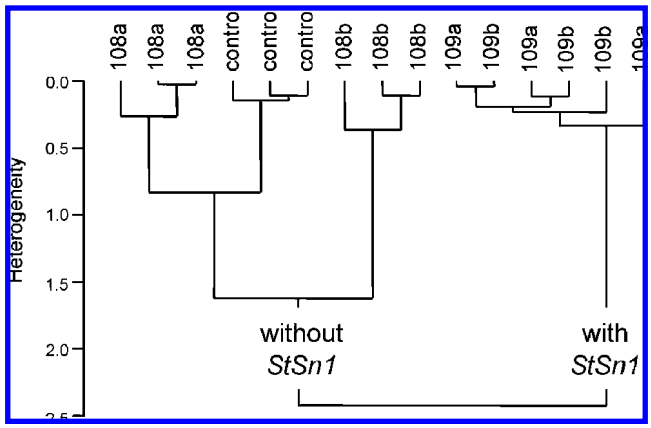

Figure 5. Dendrogram showing classification of fresh tobacco leaves into two distinct groups after cluster analysis of the single spectrum at the wavenumber range 600−500 cm^{-1} using the first three factors for calculation of spectral distances and the Ward's algorithm. Control = the nontransformed plant; 108 and 109 = the genetically modified plants without and with *StSn1* gene, respectively. Two independent plants (a and b) were measured for each genetic modification and three measurements were taken from each plant.

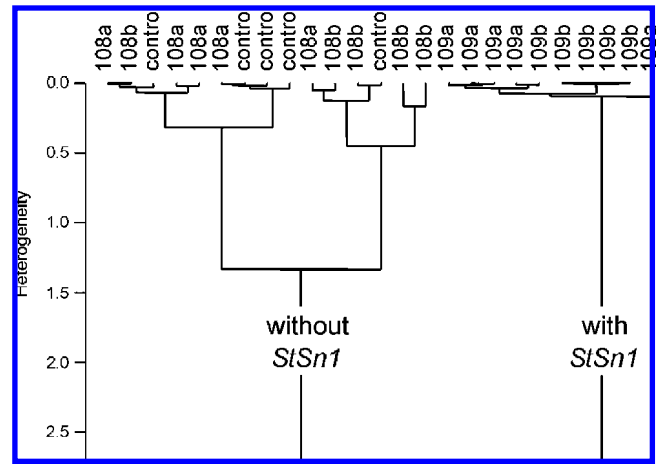

Figure 6. Dendrogram showing classification of tobacco seeds into two distinct groups after cluster analysis of the single spectrum at the wavenumber range 600−500 cm^{-1} using the first two factors for calculation of spectral distances and the Ward's algorithm. Control = seeds from the nontransformed plant; 108 and 109 = seeds from genetically modified plants without and with *StSn1* gene, respectively, and collected from two independent plants (a and b). Five measurements were taken from each seed lot.

Specific structure elements such as the peptide S−S group of cysteine provide helpful information for a reliable interpretation of the registered Raman spectra. Identification of compounds containing disulphide bonds can be successfully obtained by using FT-Raman spectroscopy because the S−S stretching band is polarized and prominent in the Raman spectra (*22*). Additionally, the conformational study of the disulphide bridge can be performed, since this group may occur in three different conformations. As could be seen in the Raman spectra of rice globulin, the disulphide bonds of cysteine residues were detected in the wavenumber range between 510 and 540 cm^{-1} (*23*). The major conformation was *gauche-gauche-gauche* as indicated by the Raman band at 512 cm^{-1}, which is the most preferred conformation in many proteins with S−S bridges (*24*). Other minor bands seen at 525 and 540 cm^{-1} were assigned to *gauche-gauche-trans* and *trans-gauche-trans* conformations, respectively. These reports show that peptides containing the S−S group may be detected among other structure elements by using Raman spectroscopy. Therefore, the presence of additional S−S bonds in the GM samples could explain the differences observed between the GM and non-GM spectra in a low wavenumber range. Indeed, the GM carrot and tobacco plant material used here contained the potato *StSn1* gene coding for a cysteine-rich snakin-1 polypeptide. Snakin-1 is a small 6.9 kDa polypeptide containing 12 cysteine groups, which constitute for 19% of the amino acid chain. Sulfur atoms present in cysteines form six S−S bridges stabilizing the molecule structure (*13, 25*).

The structure and group composition in the obtained dendrograms from independent cluster analyses of spectra collected from carrot callus, tobacco leaves, and tobacco seeds confirm the effect of additional S−S bonds as the principal factor discriminating the GM and non-GM materials. Carrot callus samples separated into two distinct groups, one containing the nontransformed control and GM callus no.160 and the second group containing the remaining GM samples (**Figure 4**). The appearance of no. 160 together with the control resulted from the fact that although no. 160 was transformed, genetic rearrangements occurred within T-DNA, and the *StSn1* gene was not introduced into carrot genome, as shown above by DNA and RNA analyses. In the case of tobacco leaves, *StSn1* containing materials (plants 109a and 109b) were separated from the nontransformed control. Moreover, the control grouped together with the GM material (plants 108a and 108b) developed after transformation using plasmid containing *chit36* instead of the *StSn1* gene (**Figure 5**). This indicates that the main grouping factor was related to the presence of snakin-1 and not to the process of transformation itself. The output from cluster analysis performed for tobacco seeds was analogous to that for tobacco leaves (**Figure 6**). The distribution of GM seeds in this dendrogram is clearly restricted to one big cluster together with the nontransgenic control, indicating the lack of distinguishable changes in transgenic material unless snakin-1 is expressed. This is additionally confirmed by the fact that, in the obtained dendrograms, some GM samples expressing the *nptII* gene grouped together with the nontransformed control. In the case of carrot, it was callus no. 160 and, in the case of tobacco, plants 108a and 108b. As shown earlier, all samples possessing the *StSn1* gene contained also a functional *nptII* gene. Thus, formation of two distinct clusters, with and without *StSn1*, after Raman measurements was not related to genetic modification caused by the *nptII* gene.

Seeds produced by transgenic plants nos. 109a and 109b grouped separately from those produced by the nontransformed control plants and plants transformed with plasmid not containing the *StSn1* gene. A separate cluster of GM seeds expressing snakin-1 was achieved despite not all seeds being transgenic, which was revealed by the germination test in the presence of kanamycin. This discrepancy can be explained by the fact that the Raman measurements were taken not from a single seed but from a seed lot. As tobacco seeds are very small (below 0.4 mm in diameter), the Raman signal could be collected from several neighboring seeds simultaneously. The usefulness of Raman spectroscopy to seed analysis was reported previously (*26*). Raman spectra were obtained from germinating rape grains of conventional cultivar 'Drakkar' and from the isogenic GM line with modified fatty acid composition. By using cluster analysis applied to those data, it was possible to distinguish both seed types. Also, the Raman measurements of single seeds of various *Apiaceae* species enabled the correct separation of the accessions according to their taxonomical classification (*27*). Moreover, it was shown that Raman measurements had no

adverse effect on carrot seed, which later germinated and developed into normal seedlings. In light of these reports and the results presented here, Raman spectroscopy may be a potential tool for detection of single GM seeds without their destruction. This challenging approach requires however further experiments focused on various species and introduced genetic modifications.

The presented results show that genetic rearrangement in the plant genome resulted from the integration and expression of foreign genes can be detected by using Raman spectroscopy. However, the discrimination between the GM material and non-GM control was possible only when the GM tissue was capable of synthesizing a new cysteine-rich polypeptide that is related to a high sensitivity of Raman spectroscopy to the S−S stretching vibration. The major advantage of this technique for the discrimination of GM material is its nondestructive effect on plant tissue. Therefore, in our experiments, information on genetic status was achieved without the need of sample disruption. This nondestructive approach may be particularly valuable for the detection of GM seeds containing snakin-1, which after the measurement could be used for plant production.

ABBREVIATIONS USED

GM, Genetically modified; GMO, GM organism; FT, Fourier transform; IPI, isopentenyl pyrophosphate isomerase; MS, Murashige and Skoog medium; NPTII, neomycine phosphotransferase; *StSn1*, snakin-1 gene.

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