

Ultrasensitive detection of genomic DNA from apple leaf tissues, using surface-enhanced Raman scattering

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Abstract. Ultrasensitive detection of nucleic acids is demonstrated through exploiting the effect of surface-enhanced Raman scattering (SERS). In this work the SERS spectra of eight genomic DNAs from leaves of apple trees grown in the field (*Malus domestica* Borkh., Fam. *Rosaceae*, cultivars. Florina, Idared, Rebra, Goldrush, Romus 3, Romus 4 and the rootstocks M9 and M26) have been analyzed in the wavenumber range 200–1800 cm⁻¹. SERS signatures, spectroscopic band assignments and structural interpretations of these plant genomic DNAs are reported. SERS spectra of nucleic acids are compared here with caution, because these signals are time-dependent and are strongly influenced by DNA amount in the measured sample volume. Similarities of the SERS spectra of genomic DNAs extracted from apple leaves of Goldrush, Rebra and Florina cultivars, respectively, have been detected. Besides, the SERS spectra, corresponding to DNA from M9, Romus 4 and M26, leaves, respectively, show similar features and well resolved bands. Based on this work, specific plant DNA-ligand interactions or DNA structural changes induced by plant stress conditions associated with their natural environment, might be further investigated using surface-enhanced Raman spectroscopy.

Keywords: Surface-enhanced Raman scattering (SERS), genomic DNA, apple leaves

1. Introduction

Sensitive and detailed molecular structural information plays an increasing role in molecular biophysics and molecular medicine [16]. Therefore, vibrational spectroscopic techniques, such as Raman scattering, which provide high structural information content are of growing interest in biophysical and biomedical research [16]. However, normal Raman spectroscopy has an extremely small scattering cross section, which limits its use as a low-level bioanalytical sensor [32,38].

Recent advances in the field of surface-enhanced Raman spectroscopy have demonstrated the utility of this technology in diverse research disciplines, ranging from surface chemistry and electrochemistry to the development of protein/nucleic acid sensor technologies [14].

Any molecule located within several nanometers to the metal nanoparticle surface can contribute to the measured SERS vibrational spectrum, leading to complex spectra that can be difficult to analyze and

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interpret [36]. The main limitation of SERS spectroscopy is that it is a surface based technique, requiring the use of an appropriate surface to provide the desired enhancement [11].

To describe the overall SERS effect, long-range electromagnetic effect (EM) and short-range chemical effect (CM) [32] have been generally accepted as two separated mechanisms. Strongly increased Raman signals can be obtained due to resonances between optical fields and the collective oscillations of the free electrons in the metal [16]. The CM mechanism stems from the interaction between adsorbed molecules and the metal surface, such as charge transfer (CT) interaction of adsorbed molecules with the metal [20,32] and references therein.

The average surface enhancement factors of noble metals range from 10^3 to 10^6 [37], but have also been reported to be as high as 10^{14} – 10^{15} for single molecule detection [14] and references therein.

Particularly, DNA SERS and SERRS (surface enhanced resonance Raman scattering) signatures might provide an objective evaluation of genetic identity of plants based on species, cultivars, or geographic origin [20].

As far as plant DNA is concerned, a previous FT-Raman study of genomic DNA from leaf tissues has been conducted by us [19]. Also, we have repeated FT-Raman experiments for other DNA molecules extracted from plant tissues, but we have observed no FT-Raman signal above the reference buffer one (data not shown). Thus, applying SERS spectroscopy for the investigation of low level DNA isolated from leaves became a demand.

In this work, the SERS spectra of eight genomic DNAs from leaves sampled from apple trees grown in the field (*Malus domestica* Borkh., Fam. *Rosaceae*, cvs. Florina, Idared, Rebra, Goldrush, Romus 3, Romus 4 and the rootstocks M9 and M26) have been studied between 200–1800 cm^{-1} . SERS signatures, spectroscopic band assignments and structural interpretations for these plant genomic DNAs are reported.

In biochemical fields, plant DNA might be used to explore the interaction between DNA and small molecules, which is important in connection with probing the accurate local structure of DNA and with understanding the natural DNA-mediated biological mechanisms [32].

2. Materials and methods

2.1. DNA extraction protocol

Leaves sampled from apple trees grown in the field (*Malus domestica* Borkh., Fam. *Rosaceae*, cvs. Florina, Idared, Rebra, Goldrush, Romus 3, Romus 4 and the rootstocks M9 and M26) were selected for our studies.

Genomic DNA was isolated from leaves of the above mentioned cultivars and rootstocks [8]. DNA extraction protocol is presented elsewhere [20].

2.2. Surface-enhanced Raman spectroscopy

2.2.1. Chemicals

Analytical reagent grade, characterized all chemicals used in this work. The silver colloidal SERS substrate was prepared by reducing Ag^+ with hydroxylamine [17]. Metallic nanoparticles with an estimated size of 34 nm have been obtained [17].

Method used for silver colloid preparation is described elsewhere [20].

Table 1
DNA amount in the measured samples volume

Apple leaves	DNA amount (ng/ μ l)
Goldrush	0.1021
Rebra	0.0881
Romus 3	0.131
Idared	0.122
M9	0.122
Romus 4	0.088
M26	0.070
Florina	0.070

Each sample was prepared by adding 500 μ l silver colloid into a 1 ml glass cuvette. Certain DNA solution quantities were added to the silver colloids. The final DNA amount in the measured samples volume is shown in Table 1. Different DNA concentrations have been analyzed by us, as compared with our previous work [20].

We did not observe any change of colour in the DNA samples prepared for SERS study, this being a proof that the silver remains colloidal and is not aggregated upon interaction with genomic nucleic acids [20].

For several samples, SERS spectra of colloidal suspensions with different DNA amount were registered. The most accurate SERS spectrum, showing well resolved bands, is presented in Fig. 1 for each DNA type sample.

2.2.2. Instrumentation

A DeltaNu Advantage spectrometer (DeltaNu, Laramie, WY, USA) equipped with a doubled frequency Nd:YAG laser emitting at 532 nm and 45 mW laser power, was used for recording the SERS spectra.

These spectra were obtained by averaging 5 recordings, each of 10 s exposure.

3. Results and discussion

SERS spectral profiles in the region 200–1800 cm^{-1} of eight genomic DNAs, are presented in Fig. 1. Nucleic acids were extracted from leaves sampled from apple trees grown in the field (*Malus domestica* Borkh., Fam. *Rosaceae*, cvs. Florina, Idared, Rebra, Goldrush, Romus 3, Romus 4 and the rootstocks M9 and M26, respectively). Labels indicate wavenumber values for the more prominent bands in each spectrum (cm^{-1} units).

Table 2 presents a detailed comparative analysis of the surface-enhanced Raman scattering spectra of the eight genomic DNAs. Proposed SERS band assignments found in the literature for similar compounds are also included. In some cases, assignments are described in terms of a specific DNA atom or functional group, which makes the related Raman band very useful for recognition of specific DNA-ligand interactions [19,28].

The SERS spectrum originates from molecules that are in close proximity to the enhanced electromagnetic field at the nanoparticle surface [36].

A strong dependence of the SERS spectra on genomic DNA amount in the sample volume has been observed by us. In this regard, Fig. 2 presents the SERS spectra of genomic DNA extracted from apple leaves of Goldrush cultivar at two different concentrations. Sample corresponding to spectrum B is

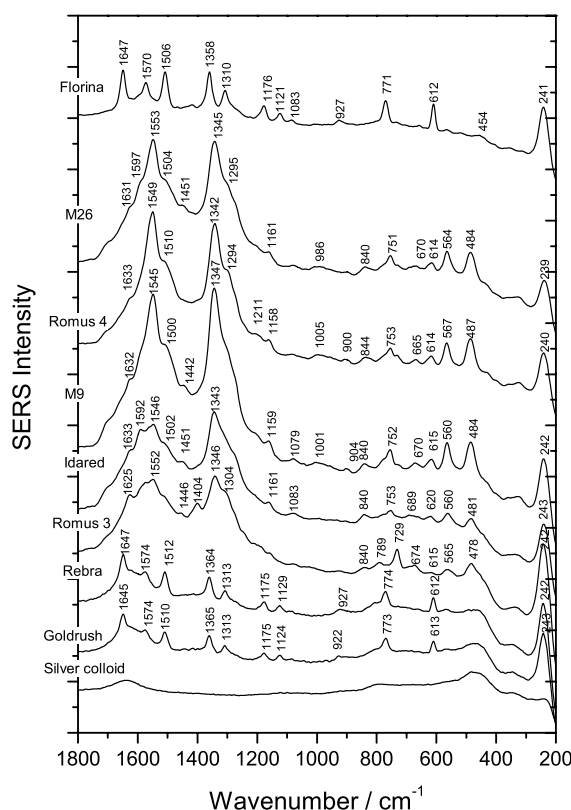


Fig. 1. SERS spectra of genomic DNAs from apple leaves sampled from the trees (cultivars and rootstocks), as labeled in the figure. Bottom, SERS spectrum of the silver colloid (blank spectrum). Spectra are presented at the same intensity scale expansion.

diluted $50\times$ as compared with the sample corresponding to spectrum A. Thus, *enhancement patterns detected in the SERS spectra are not only related to the type of DNA under consideration, but also to the packing density at the silver nanoparticles surfaces*. Previously, a correlation of molecular orientation and packing density in a dsDNA self-assembled monolayer was observed with surface-enhanced Raman spectroscopy [2]. Other authors have also found a concentration dependence of the SERS spectra of DNA molecules [12].

Besides, the well-known SERS saturation effect that occurs when too much material is on the substrate, might explain some results on DNA samples at high biomolecular concentration.

Intensity and wavenumber changes of the SERS bands of the DNA-silver colloid system with time were also observed. Figure 3 presents the SERS spectra of genomic DNA extracted from apple leaf tissues of Florina cultivar. Spectrum B was registered on the same sample as spectrum A, but one day after. Such effects have also been found by Ke et al. [15]. After several hours of interaction between the Ag colloid and the DNA, more accurate spectra were observed, with well defined SERS bands and a better signal to noise ratio.

Figure 1 shows a typical blank SERS spectrum of the silver colloid, obtained with our setup. It can be observed that the water band at $\sim 1650\text{ cm}^{-1}$ and the glass cuvette fluorescence band in the $400\text{--}800\text{ cm}^{-1}$ region, have no significant contribution in the DNA SERS spectra.

Table 2

SERS wavenumbers (cm^{-1}) and tentative assignments of genomic DNA from leaves of different apple cultivars and rootstocks, sampled from apple trees grown in the field [15,19,22,32,37,38]

Goldrush	Rebra	Romus 3	Idared	M9	Romus 4	M26	Florina	Tentative assignment ^a
		674	689	670	665	670		dG
		729						dA
			753	752	753	751		dT
773	774						771	dC
		789						dC, bk ^c
		840	840	840	844	840		$\nu(\text{O-P-O})$, B-type
				904	900			deoxyribose
922	927						927	deoxyribose
				1001	1005	986		$\nu(\text{C=C})_{\text{sym}}$ (ring), deoxyribose
			1083	1079			1083	PO_2^- sym. stretch, bk ^c
1124	1129						1121	dA
			1161	1159	1158	1161		deoxyribose-phosphate
1175	1175						1176	ring vibrations of dC,
					1211 sh ^b			dC, dT
1313	1313	1304 sh ^b			1294 sh ^b	1295 sh ^b	1310	dA
		1346	1343	1347	1342	1345		dG, dA
1365	1364						1358	dT, dA
		1404						dT, dA, dG
		1446 sh ^b	1451	1442		1451		deoxyribose, dCH ₂
1510	1512		1502 sh ^b	1500 sh ^b	1510 sh ^b	1504 sh ^b	1506	dA
		1552	1546	1545	1549	1553		dG, dA
1574	1574		1592				1570	dG, dA
1645	1647	1625	1633 sh ^b	1632 sh ^b	1633 sh ^b	1631 sh ^b	1647	dT(C=O), $\delta(\text{H}_2\text{O})$

Notes: ^a Abbreviations: dA – deoxyadenosine; dG – deoxyguanosine; dC – deoxycytidine; dT – thymidine; ^b sh – shoulder; ^c bk – backbone.

Further, in the SERS spectra of DNA presented in Fig. 1, a strong intensity band is to be observed around 241 cm^{-1} . This vibration might be assigned to $\nu(\text{Ag-N})$ or to metal-oxygen single bonds [1]. Metal-nitrogen wavenumbers, $\nu(\text{M-N})$, of single bonds were found to appear in the range between $500\text{--}200 \text{ cm}^{-1}$ [1]. Stereochemistry and coordination number, nature of the metal and its oxidation state are factors influencing the position of the wavenumber. The nature and the structure of the ligand containing the nitrogen atom to which the metal is linked, plays also an important role and influences the wavenumber [1].

The spectral interval $600\text{--}800 \text{ cm}^{-1}$ contains Raman bands that originate from vibrations involving concerted ring stretching motions (ring breathing) of purine or pyrimidine residues, often in combination with stretching of the glycosidic bond and possibly also stretching of bonds within the linked deoxyribose ring [6,22]. These bands thus combine the relatively high Raman intensity typical for base residue vibrations with the sensitivity in wavenumber value expected from changes in glycosyl torsion angle and/or deoxyribose ring pucker ($\text{C2}'\text{-endo}$ vs. $\text{C3}'\text{-endo}$) [6,22].

Bands appearing in the SERS spectra of genomic DNAs from leaf tissues between 665 and 674 cm^{-1} (see Fig. 1) are supposed to be the diagnostic of the $\text{C2}'\text{-endo-syn}$ conformation of dG [30]. Band near 689 cm^{-1} , in the case of DNA extracted from Idared cultivar, is a diagnostic of $\text{C2}'\text{-endo-anti}$ conformation of dG. However, it is possible that the wavenumber shifts observed for these peaks in the

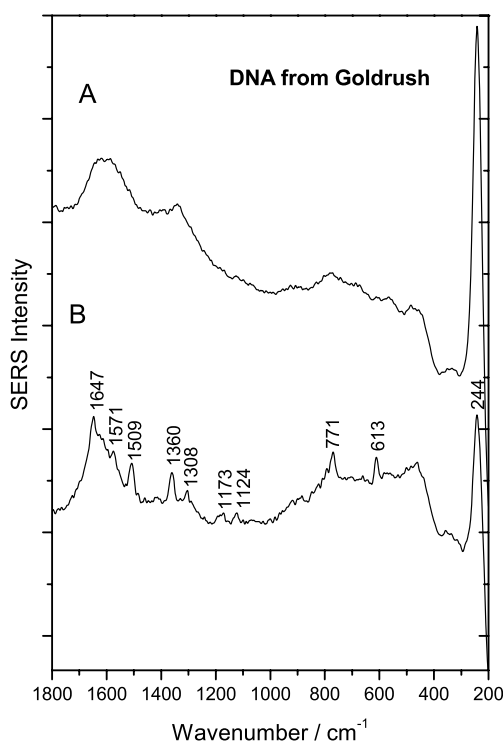


Fig. 2. SERS spectra of genomic DNA extracted from apple leaves of Goldrush cultivar. Sample corresponding to spectrum B is diluted 50 \times as compared with the sample corresponding to spectrum A. Spectra are presented at the same intensity scale expansion.

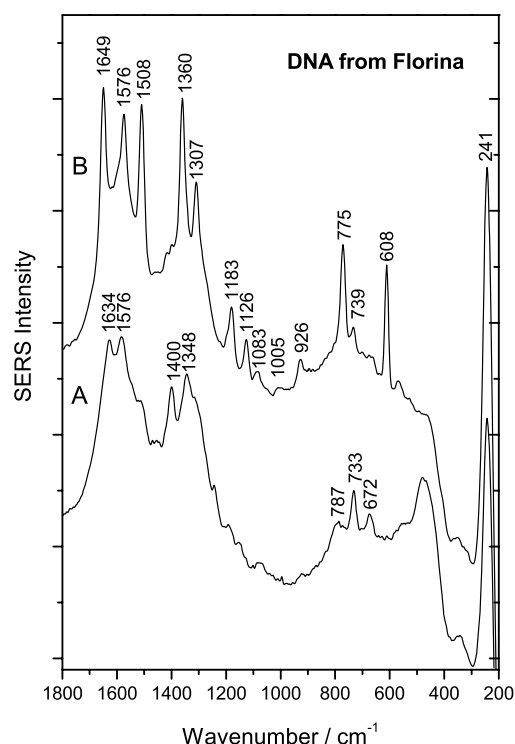


Fig. 3. SERS spectra of genomic DNA extracted from apple leaf tissues of Florina cultivar. Spectrum B was registered on the same sample as spectrum A, but one day after. Spectra are presented at the same intensity scale expansion.

SERS spectra as compared with previous normal Raman literature, might be due to the interaction of the metallic substrate with DNA molecules [32]. A partial conformational change upon adsorption of the biomolecule to the silver surface might be considered to take place.

A band noticed at 729 cm^{-1} in the case of DNA from Romus 3 cultivar, might be due to the C2'-*endo*-anti nucleoside conformers of dA [7,21,22,27,33] (see Table 2).

The dT conformation marker was observed near 752 cm^{-1} for DNA extracted from tissues of M9 leaves. The same SERS band shifted to 753 cm^{-1} in the case of DNA extracted from Idared and Romus 4 leaves, respectively, and to 751 cm^{-1} for leaf tissues of M26 rootstock.

A strong band was found at 773 cm^{-1} for genomic DNA extracted from Goldrush leaves (Fig. 1). This band, originating from C2'-*endo*-anti nucleoside conformers of dC, appeared at 774 cm^{-1} in the SERS spectrum of DNA from Rebra cultivar and at 771 cm^{-1} for genomic DNA extracted from cv. Florina leaves. In the case of the band near 789 cm^{-1} in the SERS spectrum of DNA from cv. Romus 3 leaves, it is supposed that the dC ring breathing mode at 783 cm^{-1} might overlap with the backbone mode near 792 cm^{-1} [37].

The marker band of B-form DNA backbone and C2'-*endo* sugar conformations [27,30] is centered around 835 cm^{-1} [23,27,29,30] in the normal Raman spectrum of calf thymus DNA. This medium intensity band is due to a complex vibrational mode involving the deoxyribose-linked phosphodiester network (5'C-O-P-O-C3') of B-DNA [6,22].

In our SERS spectra a weak band appeared at 840 cm^{-1} for DNAs extracted from Romus 3, and Idared cultivars, and M9, M26 rootstocks leaves, respectively. The same marker band, was observed at 844 cm^{-1} for DNA sample purified from leaves of Romus 4 cultivar.

The band near 904 cm^{-1} , which was observed in the spectrum of DNA from M9 leaf tissues, is assigned to deoxyribose [10]. This band was detected at 900 cm^{-1} for genomic DNA extracted from leaves of Romus 4 cultivar.

The sugar residues of DNA are expected to be the major contributors to the weak SERS band observed near 927 cm^{-1} , in the case of DNAs extracted from cvs. Rebra and Florina leaves, respectively [19] and references therein. This band, assigned to a vibration localized in the deoxyribose moiety, was detected at 922 cm^{-1} in the case of genomic DNA extracted from leaves of cv. Goldrush (Fig. 1).

A weak band near 1001 cm^{-1} , in the SERS spectrum of genomic DNA extracted from M9 leaves is observed at 1005 cm^{-1} for cv. Romus 4 DNA, and at 986 cm^{-1} in the SERS spectrum of the DNA extracted from rootstock M26, respectively, being probably due to a $\nu(\text{C}=\text{C})_{\text{sym}}$ (ring) vibration [38]. A sharp SERS peak near 998 cm^{-1} , attributed to the deoxyribose vibration, was observed by other authors for single-stranded oligonucleotides [26].

A band is observed at 1083 cm^{-1} in the SERS spectra of genomic DNAs extracted from leaves of Idared and Florina cultivars, respectively. This mode is due to the localized symmetric stretching vibration of the phosphodioxy (PO_2^-) moiety, being independent of the base composition and sequence [4,9,13,18,23–25,34,35,37]. Also, this marker band, which has a higher intensity in the normal Raman spectrum of DNA, was found at 1079 cm^{-1} in our SERS spectrum of DNA extracted from rootstock M9 leaves (Fig. 1).

Raman spectrum of DNA contains many overlapping bands in the region $1100\text{--}1600\text{ cm}^{-1}$, which originate primarily from in-plane vibrations of base residues [6,19] and references therein. Previous studies [6] and references therein showed that these bands cannot be assigned unambiguously because of the peaks overlap and also because the intensity of the bands in this spectral range is in general sensitive to the base-stacking interactions (Raman hypochromism) [19].

The 1124 cm^{-1} band in the SERS spectrum of DNA from cv. Goldrush, might be attributed to the ring vibration of adenine [5,19]. This vibration was observed at 1129 cm^{-1} in the case of DNA extracted from Rebra leaves, and at 1121 cm^{-1} for genomic DNA extracted from cv. Florina leaf tissues.

A vibration originating mainly in the deoxyribose-phosphate appeared also in our spectra at 1159 cm^{-1} , in the case of DNA extracted from leaves of rootstock M9. This band was detected at 1158 cm^{-1} for DNA from Romus 4 cultivar and near 1161 cm^{-1} for Idared and M26, respectively.

Besides, we have found a band centered near 1175 cm^{-1} , in the SERS spectra of genomic DNA from Goldrush and Rebra cultivars, respectively, containing a major contribution from cytosine residues. This band shifted to 1176 cm^{-1} in the SERS spectrum of DNA extracted from leaves of Florina cultivar.

The SERS bands appearing in the wavenumber range $1294\text{--}1313\text{ cm}^{-1}$ for genomic DNAs from different apple leaf tissues, can be attributed to the ring vibration of adenine (see Table 2).

Besides, we suppose that the peak near 1342 cm^{-1} in the SERS spectra of the DNA extracted from Romus 4 leaves, arises from the ring stretching of dA [36,38]. Other authors indicate also the guanine residues as contributors to this vibration [15,32,37]. This band shifted to 1343 cm^{-1} for DNA from Idared cultivar, to 1345 cm^{-1} for DNA extracted from rootstock M26, to 1346 cm^{-1} for DNA isolated from leaves of Romus 3 cultivar and to 1347 cm^{-1} for DNA from rootstock M9.

The high intensity peak near 1358 cm^{-1} in the SERS spectrum of DNA from Florina cultivar is mostly due to the dT and dA residues. This band was found at 1364 cm^{-1} , in the case of DNA extracted from cv. Rebra and at 1365 cm^{-1} , in the case of DNA purified from Goldrush cultivar.

The SERS band near 1404 cm^{-1} , attributed to DNA extracted from cv. Romus 3 leaves, is due to vibrations localized in the thymine, adenine and guanine rings [32].

A weak band was detected previously by us around 1423 cm^{-1} in the SERS spectrum of genomic DNA extracted from leaves of *in vitro*-grown Rebra cultivar [20]. This band indicated vibrations of the $d(2'-\text{CH}_2\delta)$ and dA molecular subgroups and is absent in the present data set.

The shoulder at 1446 cm^{-1} in the SERS spectrum of genomic DNA extracted from Romus 3 leaves is attributed to the CH_2 scissor of deoxyribose [19,28,34,35,37]. This band was detected near 1451 cm^{-1} in the case of DNAs from Idared and M26 leaves, respectively and around 1442 cm^{-1} for genomic DNA extracted from the leaf tissues of rootstock M9. Relating to these bands, Wu and coworkers [37] concluded for some oligonucleotides, that the observations of the methylene scissoring modes of deoxyribose at 1416 and 1464 cm^{-1} , in strong to medium intensities, support the model that DNA lies flat with the backbone close to the metal surface [37].

A prominent band was found at 1510 cm^{-1} , in the spectrum of DNA extracted from Goldrush leaves, being probably due to contributions from adenine residues [20]. It appeared as a shoulder in the SERS spectrum of DNA from Romus 4 cultivar. This band shifted to 1512 cm^{-1} for DNA extracted from leaf tissues of Rebra cultivar and was detected near 1500 , 1502 , 1504 and 1506 cm^{-1} for DNAs extracted from leaves of M9, Idared, M26 and Florina, respectively.

The strong band near 1574 cm^{-1} in the SERS spectra of genomic DNA extracted from leaf tissues of Goldrush and Rebra cultivars, arises from dG and dA ring stretchings [37] and references therein. The dA vibration around this wavenumber involves a large amplitude on N6H_2 [37] and references therein. This band was found near 1570 cm^{-1} for DNA extracted from cv. Florina leaves, and at 1592 cm^{-1} for DNA from leaf tissues of cv. Idared. Other bands found between 1545 and 1553 cm^{-1} for genomic DNAs extracted from leaves of different apple cultivars and rootstocks, were also attributed to dG and dA residues (see Table 2).

The carbonyl stretching vibrations of dT, dG and dC are expected to be in the region of 1600 – 1750 cm^{-1} . The C4=O and C2=O of dT lead to strong peaks at 1652 and 1672 cm^{-1} , C6=O of dG gives a moderate intensity peak at 1713 cm^{-1} , and C2=O of dC near 1680 cm^{-1} is extremely weak in the normal Raman spectra [20,37] and references therein. We have observed a strong peak around 1647 cm^{-1} in the SERS spectra of DNA extracted from leaf tissues of Rebra and Florina cultivars, which might be due to C=O stretching vibration of dT. This band appeared at 1645 cm^{-1} in the SERS spectrum of DNA from Goldrush, at 1625 cm^{-1} for DNA from Romus 3 cultivar and was detected as a shoulder in the case of Idared (1633 cm^{-1}), M9 (1632 cm^{-1}), Romus 4 (1633 cm^{-1}) and M26 (1631 cm^{-1}) leaves, respectively (Fig. 1).

Many of the observed peaks show wavenumber shifts in the SERS spectrum as compared with previous normal Raman literature [32]. In a surface-enhanced Raman scattering experiment the DNA molecules may interact with the metallic substrate, resulting a wavenumber shift of the Raman band [32]. Also, intensity changes with regard to normal Raman spectra of DNA molecules can be appreciated. An example of the relative peak intensities in the normal Raman and SERS spectra of oligonucleotides is given elsewhere [37].

Some authors have found that SERS features of DNA appeared to be sequence- and/or composition-dependent [3] and references therein. SERS spectra of nucleic acids-silver colloid systems are compared here with caution, because these signals are time-dependent and are strongly influenced by DNA amount in the measured sample volume. So, the relative orientation of the molecule with respect to the metal surface, which in turn is dependent on the biopolymer concentration, is also responsible for the observed SERS signal [26].

Similarities of the SERS spectra of genomic DNAs extracted from leaves sampled from apple trees grown in the field, in the case of Goldrush, Rebra and Florina cultivars, respectively, have been found (Fig. 1 and Table 2). These SERS spectra show well resolved, accurate bands, providing thus a high molecular structural information content. Besides, another group of three SERS spectra, corresponding to DNA from M9, Romus 4 and M26 leaves, respectively, show similar features and well resolved bands (Fig. 1).

The present SERS data are much better as compared with our previous FT-Raman spectra obtained for DNA from different sources of leaf tissues, as far as band resolution and signal to noise ratio are concerned [19].

4. Conclusions

In this work, surface-enhanced Raman spectra of eight genomic DNAs from apple leaves sampled from trees grown in the field (*Malus domestica* Borkh., Fam. *Rosaceae*, cvs. Florina, Idared, Rebra, Goldrush, Romus 3, Romus 4 and the rootstocks M9 and M26) were recorded and discussed, respectively, in the 200–1800 cm^{-1} spectral range.

A detailed comparative analysis of the SERS signatures of genomic DNAs extracted from apple leaves is given. SERS wavenumbers (cm^{-1}) are reported here for all types of vibrations of plant genomic DNAs, including bands assigned to localized vibrations of the purine and pyrimidine residues, localized vibrations of the deoxyribose-phosphate moiety, etc. [6,19]. Proposed SERS band assignments found in the literature for similar compounds were given.

Strong time dependence of the SERS spectra, and dependences on genomic DNA amount in the sample volume have been found. That is why SERS spectra of nucleic acids are compared here with caution.

Bands near 689 cm^{-1} (dG), 729 cm^{-1} (dA), 752 cm^{-1} (dT), 771 cm^{-1} (dC), 840 cm^{-1} [$\nu(\text{O-P-O})$, B-type], 904 cm^{-1} (deoxyribose), 927 cm^{-1} (deoxyribose), 1005 cm^{-1} [$\nu_{\text{C}=\text{C}}^{\text{sym}}$ (ring), deoxyribose], 1083 cm^{-1} (PO_2^- symmetric stretch, backbone), 1124 cm^{-1} (dA), 1159 cm^{-1} (deoxyribose-phosphate), 1175 cm^{-1} (dC), 1304 cm^{-1} (dA), 1342 cm^{-1} (dG, dA), 1358 cm^{-1} (dT, dA), 1404 cm^{-1} (dT, dA, dG), 1451 cm^{-1} (deoxyribose, dCH_2), 1510 cm^{-1} (dA), 1574 cm^{-1} (dG, dA) and 1647 cm^{-1} [$\text{dT}(\text{C}=\text{O})$, $\delta(\text{H}_2\text{O})$], characteristic to genomic DNAs from different leaf tissues, sampled from trees grown in the field, are presented in detail.

In the case of Goldrush, Rebra and Florina cultivars, respectively, similarities of the SERS spectra of genomic DNAs extracted from leaves of apple trees, have been found. Besides, the SERS spectra, corresponding to DNA from M9, Romus 4 and M26, leaves, respectively, show similar features and well resolved bands.

The SERS technique-based data presented here will contribute to the rapidly growing field of nanometrology and bioanalysis using silver nanoparticles [31].

Once spectra-structure correlations in the SERS spectra of apple leaf DNAs being established in our work, future SERS experiments on plant DNA can be carried out to analyze specific genomic DNA-ligand interactions.

Besides, applications of plant DNA vibrational markers in forensic botany might be anticipated.

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