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Surface-enhanced Raman spectroscopy of genomic DNA from *in vitro* grown tomato (*Lycopersicon esculentum* Mill.) cultivars before and after plant cryopreservation

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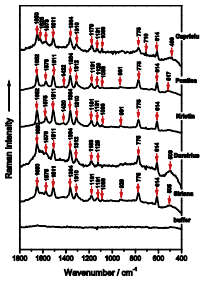
HIGHLIGHTS

- Structural characteristics of genomic DNAs from tomato cultivars were analyzed.
- Structural changes induced in genomic DNAs upon cryopreservation were discussed.
- Cultivar dependent influence of cryogenic storage on DNA structure has been observed.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work the surface-enhanced Raman scattering (SERS) spectra of five genomic DNAs from non-cryopreserved control tomato plants (*Lycopersicon esculentum* Mill. cultivars Siriana, Darsirius, Kristin, Pontica and Capriciu) respectively, have been analyzed in the wavenumber range 400–1800 cm^{-1}. Structural changes induced in genomic DNAs upon cryopreservation were discussed in detail for four of the above mentioned tomato cultivars. The surface-enhanced Raman vibrational modes for each of these cases, spectroscopic band assignments and structural interpretations of genomic DNAs are reported. We have found, that DNA isolated from Siriana cultivar leaf tissues suffers the weakest structural changes upon cryogenic storage of tomato shoot apices. On the contrary, genomic DNA extracted from Pontica cultivar is the most responsive system to cryopreservation process. Particularly, both C2′-*endo*-anti and C3′-*endo*-anti conformations have been detected. As a general observation, the wavenumber range 1511–1652 cm^{-1}, being due to dA, dG and dT residues seems to be influenced by cryopreservation process. These changes could reflect unstacking of DNA bases. However, not significant structural changes of genomic DNAs from Siriana, Darsirius and Kristin have been found upon cryopreservation process of tomato cultivars. Based on this work, specific plant DNA–ligand interactions or accurate local structure of DNA in the proximity of a metallic surface, might be further investigated using surface-enhanced Raman spectroscopy.

Introduction

The technique of surface-enhanced Raman scattering (SERS) is a powerful and promising analytical tool, which is undergoing rapid

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development in biomedical fields, because it can sensitively and selectively recognize biomolecules such as DNA/RNA, proteins and can analyze medical specimens such as bacteria and drugs, based on their spectral fingerprint characteristics ([1] and references therein).

As compared with normal Raman signals, SERS scattering cross-sections of molecules residing at or near the surface of roughened or nanostructured materials may be enhanced by factors up to $\sim 10^{14}$ or close to that of fluorescence ([2] and references therein).

Particularly, the enhancement mechanism for surface-enhanced Raman scattering comes from intense localized fields arising from surface plasmon resonance in metallic (e.g. Ag, Cu, Au) nanostructures with sizes in the order of tens of nanometers [3]. Besides, the charge transfer (CT) mechanism is associated with a charge transfer process between the adsorbed analyte and the metal surface ([4] and references therein). Therefore, vibrations involved in the charge transfer process are enhanced ([5] and references therein).

Particularly, DNA SERS signatures might provide an objective evaluation of genetic identity of plants based on species, cultivars or geographic origin [6,7].

In this work, SERS spectra of genomic DNAs isolated from leaves of *in vitro* grown tomato (*Lycopersicon esculentum* Mill. cultivars Siriana, Darsirius, Kristin, Pontica and Capriciu), in non-cryopreserved control plants, respectively, have been studied between 400 and 1800 cm^{-1}. Structural changes induced in genomic DNAs upon liquid nitrogen treatment of shoot apices were discussed in detail for four of the above mentioned tomato cultivars. SERS signatures, spectroscopic band assignments and structural interpretations for these plant genomic DNAs are reported. Applying an ultrasensitive Raman method for these low level DNA samples became a demand [6,8].

Tomato is a diploid species with 12 chromosomes and a genome size of approximately 950 Mb [9]. Worldwide, tomato represents a major horticultural crop, playing an important role in human diet. Tomatoes are a convenient source of different classes of antioxidants such as carotenoids, ascorbic acid, phenolic compounds and α-tocopherol [10]. Lycopene, one of the most powerful natural antioxidants, is the most abundant carotene in red tomato fruits, accounting for up to 90% of the total carotenoids. These compounds have a high positive impact in human health, so that tomato has been pointed as a functional food. Therefore, tomato consumption has been associated with decreased risk of breast, head and neck cancers and might be strongly protective against neurodegenerative diseases [11,12]. Tomato cultivars worldwide in tropical and temperate climate regions suffer mainly from tomato bacterial wilt disease incited by *Ralstonia solanacearum* [13] and cucumber mosaic virus [14]. For the long-term conservation of genetic resources the availability or development of reliable strategies and the subsequent regeneration of plants following storage are basic requirements [15]. Access to high diversity of genetic resources is a prerequisite for breeding novel tomato cultivars [16].

Cryopreservation is an attractive alternative for the long-term storage of healthy plant genetic resources, consisting in the conservation of plant material at ultra-low temperatures (in liquid nitrogen at −196 °C) [17]. At this temperature cell divisions and all other biological activities are completely arrested. The transfer of cells from room temperature to −196 °C must be done in such a way that the viability of the stored material is retained, so that their biological functions and growth can be reactivated after thawing and transfer to the regrowth medium [18]. Vitrification based procedures are particularly suitable for cryopreservation of complex tissues such as shoot tips. Since the development of the plant vitrification solution [19], cryopreservation by vitrification with PVS2 has been successfully used for the long-term storage of numerous herbaceous or woody plant species [20,21]. It is essential to underline the complementarity of *in vitro* techniques with cryopreservation as important tools for the *ex situ* conservation and management of plant genetic resources [22].

Plant DNA might be further used to explore particular DNA–ligand interactions ([23] and references therein), in connection with probing the accurate local structure of DNA near a metallic surface and with understanding the natural DNA-mediated biological mechanisms.

Materials and methods

Cryogenic storage and DNA extraction

For initiation of *in vitro* cultures of tomato (*L. esculentum* Mill., cultivars Siriana, Darsirius, Kristin, Pontica and Capriciu), seeds were surface sterilized with 75% Clorox (active chlorine content 5%) for 15 min and rinsed three times with sterile distilled water. The seeds were germinated in 200 ml Erlenmeyer flasks on half-strength (1/2) MS [24] medium supplemented with 30 g l^{-1} sucrose and solidified with 7.6 g l^{-1} agar; the pH of the medium was adjusted to 5.7 prior autoclaving at 120 °C for 20 min. The cultures were grown at 22 ± 1 °C and 16 h light/8 h dark photoperiod using cool white fluorescent light (36 μmol s^{-1} m^{-2} photosynthetic active radiation). Shoot apices, consisting in the meristematic dome with 2–4 leaf primordia (2–3 mm in length), were excised in sterile conditions and were transferred to 10 ml liquid MS medium enriched with 0.5 M sucrose for 24 h. Following osmoprotection in sucrose, explants were dehydrated with the plant vitrification solution 2 (PVS2) [19] for 20 min. For cryopreservation in liquid nitrogen (−196 °C), shoot apices were transferred to previously sterilized aluminum foils (0.5 × 2 cm in length) in PVS2 droplets (6 μl) and were directly immersed in liquid nitrogen. After 24 h storage at −196 °C, rewarming of samples was performed in liquid MS medium with 30 g l^{-1} sucrose (pH 5.7) at room temperature. Thereafter, explants were transferred to MS solid (with 5 g l^{-1} agar) medium in Petri dishes (5 cm diameter) for plant recovery in the same culture conditions as mentioned above.

DNA from plants regenerated following cryopreservation was compared with DNA extracted from *in vitro* grown tomato plants (non-cryopreserved controls), respectively. Genomic DNA was isolated from leaves (100 mg) of the above mentioned cultivars, 45 days after cryopreservation, using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. Extracted DNA was dissolved in 10 mM Tris–HCl, 0.5 mM EDTA, pH 9.0. Purified total DNA was quantified using the NanoDrop 2000 Spectrophotometer. DNA samples were stored at −80 °C until use.

Surface-enhanced Raman spectroscopy

Preparation of DNA–nanoparticle complexes

Analytical reagent grade chemicals were used in this work. The silver colloidal SERS substrate was prepared by reducing Ag^+ with hydroxylamine. Metallic nanoparticles with an estimated medium diameter of 34 nm have been obtained [6,25]. Briefly, 0.017 g silver nitrate were solved in 90 ml ultrapure water. In a separate recipient, 0.017 g of hydroxylamine hydrochloride were solved in 10 ml water, followed by the addition of 1.1 ml sodium hydroxide solution (1%). The hydroxylamine/sodium hydroxide solution was then added rapidly to the silver nitrate solution under vigorous stirring [4,7]. After few seconds a gray–brown colloidal solution resulted and it was further stirred for 10 min. The pH value of the silver colloid, measured immediately after preparation, was found to be 7.5. However, after one day the pH value lowered to 6.5, remaining constant at this value for the next weeks [4,7]. The pH value of all measured SERS mixtures, containing colloidal nanoparticles