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POLYACETYLENE AND CAROTENES FROM *PETROSELINUM SATIVUM* ROOT

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Polyacetylenes, carotenes and other components such as starch, pectin, cellulose, lignin were investigated in the root of *Petroselinum sativum* by a noninvasive spectroscopic method. The components were measured *in situ*, directly in the plant tissue, with no preliminary sample preparation. The analysis was done on the basis of intensity of characteristic bands observed in Raman spectrum. The principal polyacetylenes from *P. sativum* are all–cis–polyacetylene $(C_2H_2)_n$ and all–trans–polyacetylene $(C_2H_2)_n$ have similar molecular structure, but present in their spectra a change of the symmetric group –C=C–C=C– from 2210 cm^{-1} to 2002 cm^{-1}. The differences observed can be due both to the conformational differences and those existing in the environment. By Raman quantification were detected polyacetylenes that visualize the distribution of biostructures between the sections in root of *P. sativum*. The technique of quantification was applied to evaluate the distribution of some compounds such as lignin and polyglucides. Results showed an internal tissue specific accumulation in starch and biostructures like lignin, pectin and cellulose in the cell wall.

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1. Introduction

Raman spectroscopy is based on the phenomenon of scatter light in the field of UV-VIS and near IR. Raman spectroscopy is a non-disruptive technique that can be applied on solid, liquid or gas substances, but is a "weak" process, the efficient section for scatter is 10^{-31}–10^{-26} cm^2/molecule. Some plants contain aliphatic biostructures C17-polyacetylenic. Polyacetylenes are biostructures formed of a system of double and triple bonds, which proved to be toxic against fungi, bacteria and some types of mammal cells [1]. It was demonstrated their neurotoxic, anti–inflammatory and anti–platelet–aggregatory effects that might be responsible for cutaneous allergic reactions [2, 3]. The concentration of polyacetylenes in plants is of 0,01–1% order. The plants biostructures were studied by grinding whole plants followed by extraction with a mix of petroleum ether / diethylic ether and injection of solution through a chromatographic column in a UV spectrometer cell. This allowed the optimization of the extraction procedure and preparation process of pure products [4]. Thus, it was elucidated the polyacetylene structure, analyzing these biostructures and comparing observations with results of other methods of instrumental analysis.

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Polyacetylens are sensitive to light and heat, and sometimes they can be damaged during the distillation or during the process of determining the melting point. They can be stored in dark, in nitrogen [5, 6]. The function of polyacetylens in plants is not yet clear. Polyacetylenes in fungi can present antibiotic properties [7]. These biostructures are not distributed uniformly in the whole plant, but concentrated in some parts of the plant, like flowers, leaves, roots, or seeds. Distinct biostructures were quantified in different parts of the same plant. The spectra library of the natural compounds in IR and Raman obtained from more than 1500 plant samples, can be used to set the type of biocompound [8].

These spectra are combined with molecular structures and individual data. The bands observed in the region 2210 cm^{-1} in the molecular structure, correspond to the Raman spectra with acetylenic groups. All acetylenic groups are disubstituted. The most spectra of the molecules with mono- and diacetylenic groups have a strong band at 2230 cm^{-1}, especially in case of adjacent group from the triple and aliphatic bonds. Some molecules have a weak satellite band at approximately 2190 cm^{-1}, that can be due to isotopic substitution of triple bonds [9].

Biocompounds like apiol and 3,4-methylenedioxybenzonitrile (with acaricidal properties) determined by allyl functional groups [$–C_3H_5$] and methoxy [$–OCH_3$]), have indicated bands at 2191 and 2198 cm^{-1}. Raman spectrum of triacetylenes unsubstituted show two very strong bands, at 2212 and 2019 cm^{-1}, due to the elongation vibration of the triple bonds [10]. When they are exposed to abiotic stress during harvesting, transport, storing and handling, plants are capable of synthesizing biometabolites that give sour taste. The FT-Raman spectra were recorded for plants in which polyacetylenes are present especially in low concentration, along with other biostructures of the biologic mechanism of plant [11].

The nondisruptive analysis of the plant biosubstances is important in biochemistry, to explore sources of drugs and raw materials for the pharmaceutical industry. This can be achieved by FT-Raman spectroscopy with excitation at 1064 nm. Concentration and distribution of biocompounds from plants is dependent of the genotype and their taxonomy. Raman spectroscopy results help the farmers in selection of quality descendants [12]. This spectroscopy technique allows simultaneous monitoring of concentration of different vegetal substances, and based on these data it can be detected the optimum moment of harvest [13, 14].

The quantification technique of the Raman spectra over big plant parts or the entire plant has practical importance. This presents the distribution of biostructures of interest and which parts of the plants contain the biggest concentration. Raman technique allows quantitative analysis in a short time, replacing the usual procedures of separation, time-consuming.

Content of carotenes is usually determined using UV-VIS spectrophotometry at 446 nm. In this study, a spectroscopic technique, was used to identify the content of polyacetylenes and carotenes in roots of *P. sativum*.

2. Materials and methods

P. sativum was harvested from the Timişoara area at the intersection of the 45°47' parallel north latitude, with the 21°17" meridian east longitude, being, as topographic position, in the north hemisphere, at distances almost equal from the north pole and equator and in the east hemisphere, in the time zone of Central Europe, at a median distance of approximate 550 km from the capital of Romania – Bucharest and cca. 170 km and 300 km from Belgrade and respectively Budapest, capitals of the neighbor countries Serbia and Montenegro and Hungary.

Raman spectra were recorded with a spectrophotometer (Prestige, Shimadzu Europa GmbH, Duisburg, Germany), with laser source of Ar with λ= 514,5 nm, power of the laser at the sample surface was 2,33 mW, and the measure field was set between 130–5000 cm^{-1}. Measurement were done with an objective of ×100, and the parameters of spectrum elevation were: exposure time of 2 seconds, accumulation time of 5 seconds, and accumulation number of 5 cycles. Resolution was 1cm^{-1}, laser beam of 1 μm^2, and network of 1800 lines/mm [15].

3. Results and discussion

Results obtained show the utility of Raman spectroscopy in investigating polyacetylenes in roots of *P. sativum*. Presence of radicals of acetylene was confirmed by the appearance of carbonyl groups of biostructures (C=O) at 1744 cm^{-1}. The two peaks of strong intensities that correspond to values 2934 and 2980cm^{-1} are attributed to methyl and methylene groups associated to polysubstituents. Strong vibrations that appear at 3280cm^{-1}, characteristic to hydroxyl groups of the native starch, are diminished in intensity after acylation, because their number decreases [16]. Intensity of band OH at 3004 cm^{-1} depends on the degree of substitution of biostructures. Vibration band of methylenic groups increases with the increase of the degree of substitution. At 1744 cm^{-1} appears the band C=O of the polyacetylenic ester. Signals at 1420 cm^{-1} and 1410 cm^{-1} and those at 1100–907 cm^{-1} are attributed to vibrations C–O–C and association vibrations C–O–C in the polyacetylenic skeleton.

The peak δC–H appears at 1360 cm^{-1} and is corresponding to acetylenes and the polyacetylenic chain of biostructures with carboxylic residues. The biostructures were quantified directly in the plant tissue, with no preliminary preparation of the sample. Polyacetylenic spectral signals of the analyzed root are weaker comparative to those of carotenoids of the same matrix. Molecular biostructures of psoralen, 8-methoxypsoralen, 5-methoxypsoralen, oxypeucedanin, and isopimpinellin; ficusin, bergapten, majudin, and heraclin, are similar, but present small Raman spectral differences demonstrated by the shift –C≡C–. Quantification of the line done along the transversal diameter cut in roots of *P. sativum* were used in order to investigate the relative quantity of polyacetylenes and carotenes. An image of the area presented in Figure 1. offers information regarding the distribution of both types of biocompounds [17].

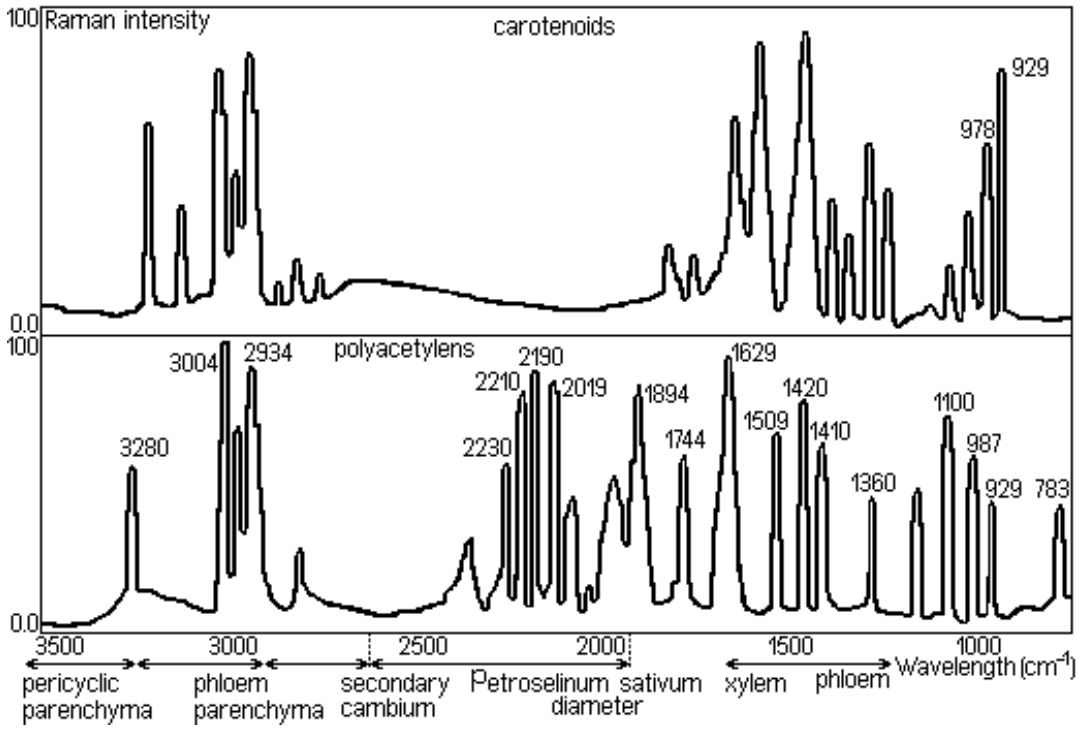

Fig. 1. Spectral responses of biocompounds from the P. sativum root

It was observed a high accumulation of polyacetylenes in the exterior section of the root, especially the pericyclic parenchyma, and another quantity in floem close to the secondary cambium. The highest quantity of carotene is quantified in the close vicinity of polyacetylene conglomerates. Calibration curves varying from 200-800 ppm were prepared by extraction of carotene from *P. sativum* [18]. Calibration models were developed to quantify carotene in the

spectral field 987–929 cm^{-1} for spectroscopy [19, 20]. Due to their rapidity and simplicity, the spectroscopic techniques, offer alternative means of quantification of the content of carotene in roots of *P. sativum*. More than that, this spectroscopic technique is ecologic, because it does not imply solvents.

4. Conclusions

There were recorded Raman spectra for the root of *P. sativum* by FT-Raman spectroscopy, a non-disruptive method, using radiations at 1064 nm. It was not observed a fluorescence interference. There were identified acetylenic biocompounds in root of *P. sativum*, even distinct compounds, with varying values in different parts of the root. Distribution of the different biocompounds in root of *P. sativum* could be observed, and also the modifications in the ontogenesis time can be followed by Raman quantification technique. From the library of Raman and IR spectra, the structure of the biocompounds synthesized was identified. Raman technique allows analysis in a short time, replacing the usual time consuming separation procedures, and avoid destruction of samples during the analysis procedures.

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