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Application of fluorescence spectroscopy as a field method in the determination of varietal differences after tomato harvesting

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Abstract: The study's purpose is to establish the application based on fluorescence spectroscopy as a field method in the determination of varietal differences after tomato harvesting. The tomato fruits will be compared to determine the spectral distribution due to the varietal differences of a particular genotype. This will allow the approach to be practiced non-invasively in the quality control of tomato production in unspecified rooms and outdoors.

The experimental studies have been conducted locally at the Institute of Plant Genetic Resources "K. Malkov" - Sadovo for three varieties.

The spectral installation for the generation of emission fluorescence spectra is mobile. In its adjustment (optical adjustment), a system engineering approach based on the classical principles of modern optoelectronics was applied. The results of the experiment can be used to optimize the time for the analysis of the varietal difference of tomato genotypes after harvesting, under uncontrolled conditions. This will support the process of determining the belonging of a specific accession to a given variety (even for accessions of unknown origin) when it is necessary to qualify a score of samples in a short time.

Key words: tomato fruits, uncontrolled conditions, field method, fluorescence spectroscopy

Uporaba fluorescentne spektroskopije kot metode za ugotavljanje razlik med sortami paradižnika na polju po obiranju

Izvleček: Namen raziskave je bil razviti poljsko metodo za ugotavljanje razlik med sortami paradižnika po obiranju z uporabo fluorescenčne spektroskopije. Razlike med sortami so določene na osnovi razlik med porazdelitavmi spektrov, ki so odvisni od genotipov. To bo omogočilo praktičen neinvazivni nadzor kakovosti pri pridelavi paradižnika na prostem in v nespecializiranih skladiščih. Poskusi so bili izvedeni na ustanovi Institute of Plant Genetic Resources "K. Malkov – Sadovo, Bolgarija, na treh sortah.

Naprava za generiranje emisijskega fluorescentnega spektra je mobilna. Za njeno nastavitev (optična nastavitev) je bil uporabljen klasični princip moderne optične elektronike. Rezultati te raziskave bi se lahko uporabili za optimiziranje časa analize razlik med genotipi različnih sort paradižnika po obiranju v nenadzorovanih razmerah. To bo podpora pri določanju akcesij, ki pripadajo določeni sorti paradižnika, tudi tistih neznanega izvora, kadar je potrebno pregledati kakovost vzorcev v kratkem času.

Ključne besede: plodovi paradižnika, nenadzorovane razmere, poljska metoda, fluorescentna spektroskopija

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1 INTRODUCTION

The established function of fluorescence spectroscopy as a field method in the assurance of varietal differences after tomato harvesting is the aim of the present study. Various techniques have been investigated for non-invasive spectrometric analysis of tomatoes. Near-infrared spectroscopy is used to determine the content of soluble solids (Slaughter et al., 1996) and detect carotenoids (Pedro and Ferreira, 2005) has been successfully applied. Also, reflectance approaches (Polder et al., 2004) and fluorescence spectroscopy (Lai et al., 2007) have been well enforced to assess surface pigmentation. Raman spectroscopy is a proven technique in carotenoid research (Schulz et al., 2005; Pudney et al., 2011).

In the assessment of the ripeness and firmness of tomatoes (Qin and Lu, 2008), the absorption and scattering properties are applied. Advances in fiber optic applied science attempt to provide outstanding conveniences for the development of an ample range of highly deftly fiber optic sensors in many modern application fields. Fiber optic insides have been successfully becoming assemblies with micro-optic pieces such as lenses, mirrors, prisms, gratings, and others (Dakin & Brown, 2006; Mitchke, 2010).

In many analytical areas of science, fluorescence spectroscopy is an important research tool. It is currently the dominant methodology and is widely recycled in biotechnology, flow cytometry, medical diagnostics, DNA sequencing, agriculture, and genetic investigations, as well as in many other application areas. Methods using this light phenomenon are highly sensitive and rapid and do not require the expense and difficulty of using radioactive tracers (Becker et al., 2003; Albani, 2006).

For quality control of vegetable crops, including tomatoes, the effects of light apply to spectral analysis such as fluorescence, transmission, and diffuse reflectance. Also, they can serve as a field method in the determination of varietal differences after tomato harvesting, since fluorescence emission is visualized in the visible spectral range and from ultraviolet rays. The spectral distribution of the emission signal in tomato fruits consists mainly of two maxima in the visible range. The intensity and shape of the fluorescence emission spectrum at room temperature depend mainly on the concentration of the fluorophores and to a lesser extent on the structure, photosynthetic activity, and arrangement of the cells in the tissue (National Research Council, 1968; Leo et al., 2007).

In connection with the demands of consumers for high food quality, the conducted research can serve as a basis for the creation of mobile detecting devices with which to carry out instant analysis of warehouse production of tomatoes in uncontrolled conditions, both in processing plants and in food retail outlets.

The present study aims to establish the function of fluorescence spectroscopy in the act of field method in the determination of varietal differences after tomato harvesting. They will be compared to determine the spectral distribution due to the varietal differences of a particular genotype. The specimens were grown under uncontrolled field conditions. This will permit the technique to be applied non-invasively in the quality control of tomato production in unspecified rooms and outdoors.

2 MATERIALS AND METHODS

The fruits that are the subject of the research are the varieties Local Dwarf (determinate), Pikador (determinate), and Ideal (indeterminate). The seeds that were sown to grow the accessions were taken from GenBank at the Institute of Plant Genetic Resources, Sadovo.

The local Dwarf tomato was dense, planted up to 40–50 cm tall, had potato-type leaves, bright red fruits with a flat round shape, and a mass of 100–120 g.

The determinate cultivar Pikador is a highly efficient variety, making fruit with fiercely red flesh that is considerable for preserving. Admirably, lightly stretched tomatoes that parallel pears in shape are firm and hard, with a mass of 50–60 g. The fruits of tomatoes of this variety are located in the form of a bunch on the plant. The variety is strongly resistant to mold.

The indeterminate Ideal was a medium-early tomato variety with considerable fruits, 130–180 g, flat-round to arced, kind of ridged, multi-chambered, orange-red in color, and an amiable taste.

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The experiment was conducted at the Institute of Plant Genetic Resources „Konstantin Malkov“ - Sadovo. The seeds are set in seedling plates filled with peat-pearlitic substrate and launched at a temperature of 25–28 degrees C for impregnation. Tomato seedlings are transplanted once they are 15–25 cm in height and have 3–5 true leaves.

Indeterminate ‘Ideal’ is planted at an area amid rows of 75-80 cm and 35-40 cm - betwixt plants in the row. The branches are attached to a supporting structure.

Determinate ‘Pikador’ and ‘Local Dwarf were planted 25–30 cm between tomato plants, and space rows 60–90 cm aside.

The field experiment was block design with three repetitions, with ten (10) plants in repetition.

The agrotechnical measures were carried out in the excellent terms for the crop.

Fluorescence spectroscopy was applied to determine the varietal differences after tomato harvesting under uncontrolled conditions. The spectral analysis was performed locally at the Institute of Plant Genetic Resources „Konstantin Malkov" - Sadovo.

The analysis was carried out with a fiber-optic spectrometer, which enables the formation of emission fluorescence signals from 200 nm to 1200 nm.

The unproved setup adds a laser diode (emission wavelength 285 nm, optical power 16 mW, DC) and a compact spectrometer (exemplary AvaSpec-ULS2048CL-EVO). The accessions are placed one by one on a duralumin plate. The experimental setup allows emission fluorescence signals to be detected through a Y-shaped optical fiber at 180 degrees to the sample and at a distance of 1.7 cm from it. The fruits are placed and arranged on a duralumin plate, which allows the encounter of an emission signal in perpendicular geometry at 180 degrees and at a distance of 1.7 cm by Y-shaped optical fiber. This curtails aberrations and allows the formation of an exceptional emission fluorescence signal (Fig. 1). The resolution of the spectrometer can range from 0.06 - 20 nm, with that of the setup recycled for our experiment being 0.09 nm. The useful fluorescence signal is generated in a direction that is 180° from the excitation radiation, so as not to saturate the receiver. A laser diode (LED) was used as a source because its spectral width was too small. The source is high-power and more sensitive than other commercial LEDs. It generates a continuous or pulsed light signal. It has an SMA-905 connector for connecting optical fibers and works with a 5V or 1.6A power supply. LED has a relatively wide spectral width of emission (30–40 nm) and an angular distribution of emission in the range of +/–30°. The sensitivity of the spectrometer is in the range of 200 nm to 1200 nm. The mode of operation of the AvaSpec-ULS2048CL-EVO enables both the emission spectrum and the excitation source spectrum to be recorded. The emission signal represents the spectral distribution of the signal emitted by the accession after fluorescence has occurred. The excitation spectrum represents the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength of the LED. This spectrum is represented as a function of the wavelength of the light signal incident on the photodetector in the spectrometer. Complementary metal-oxide semiconductor (CMOS) instead of conventional charge-coupled device (CCD) technology, this spectrometer owes its key advantage over others with a similar configuration to the dominant position of the CMOS detector in its design. For this particular circuit, the photodetector is of the CMOS model

Figure 1: Accustomed aspect of the experimental installation used by fluorescence spectroscopy

S9132 type. Its sensitivity is in the range of 200 nm to 1200 nm. Its resolution is $\delta\lambda = 5$ nm. S9132 was chosen because it could detect emission radiation from fruit with analogous cell morphology, biology, and chemical composition.

The laser radiation is deflected against the source and hits the sample. Afterward, the sample fluoresces, and the emission signal is conducted on a U-shaped optical fiber with a core diameter of 200 μm, a step-index of refraction, and a numerical aperture of 0.22. It sends a signal to the detector. In the spectrometer, the light signal is converted to an electrical-digital signal using a USB 2.0 wire, downloaded to a computer with AvaSoft8 software, and exported to Excel. This allows analysis, processing, and visualization of the results of the conducted research.

3 RESULTS AND DISCUSSION

The optical properties of tomato fruits are determined by their energy structure, which includes both the occupied and free energy levels as well as the energy levels of the atomic vibrations of the molecules in the crystal lattice. The achievable transitions between these energy levels, as a function of photon energy, are specific to the tomato, resulting in spectra and optical properties unique to it. Tomato fruits contain particles with sizes smaller than the wavelength of visible light. Particles in the turbid medium (such as tomato fruits) act as independent light sources, emitting incoherently, causing the samples to visibly fluoresce.

Therefore, fluorescence spectroscopy finds application for analysis in this vegetable crop. The optical parameters and spectral properties also change as a function of temperature, pressure, external electric and magnetic

fields, etc. This allows obtaining essential information about changes in the chemical and cellular morphological composition of the tomato.

The spectral distributions of tomato fruits of the Local Dwarf and Pikador varieties are presented in Fig. 2. A certain correlation is observed between them (the emission wavelength of Local Dwarf t is 425 nm; the emission wavelength of Pikador is 421 nm). Their emission fluorescence signals are close in terms of peak wavelength and signal intensity level. The emission wavelengths are due to the content of certain fluorophoric compounds in tomatoes, for example, nucleocapsid (N) proteins, lectin, some carboxyl compounds, and others. This is because both varieties are determinant tomatoes. They are close in biological and cellular morphological composition. The method of fluorescence spectroscopy is applied in this study to distinguish the fruits of these two varieties since the correlation in the spectral distribution is sufficiently distinct and distinguishable. This fact is used in this study to determine the tomato fruit belonging to a given variety.

Fig. 3 shows the spectral distributions of tomato fruits of the Local Dwarf and Ideal varieties; a significant correlation is observed between them (emission wavelength for Local Dwarf is 425 nm; emission wavelength for Ideal is 410 nm). Their fluorescence emission signals are not close and have a significant offset in wavelength localization and signal intensity level. The method of fluorescence spectroscopy can be applied to distinguish the fruits of these two cultivars because the correlation in the spectral distribution is of considerable distinctness and distinction. The method of fluorescence spectroscopy may practically be used to qualitatively resolve the belonging of fruits to a given variety.

Fig. 4 shows the spectral distributions of tomato fruits of the Pikador and Ideal varieties. A significant difference is observed between them (the emission wavelength for Pikador is 421 nm; the emission wavelength for Ideal is 410 nm). Their fluorescence emission signals are not close and have a significant offset in wavelength localization and signal intensity level. Since the differences in the spectral distribution are available for these two cultivars, the method of fluorescence spectroscopy can be applied to distinguish their fruits. The method of fluorescence spectroscopy can practically be used to qualitatively determine the belonging of fruits to a given variety.

A literature survey was conducted using similar methods. It turned out that, until now, the described experimental approach for the field method in the determination of varietal differences after tomato harvesting has not been applied internationally. This gives us reason to claim that for the first time, fluorescence spectroscopy was used in the application of fluorescence spectroscopy as a field method in the determination of varietal differences after tomato harvesting under uncontrolled conditions. The method is successfully applied to distinguish

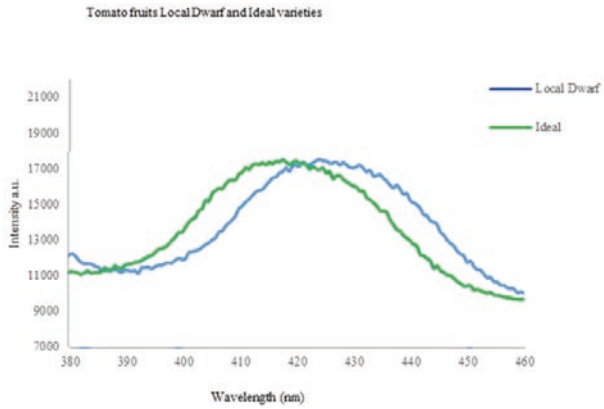

Figure 3: Emission wavelengths of tomato fruits Local Dwarf and Ideal varieties

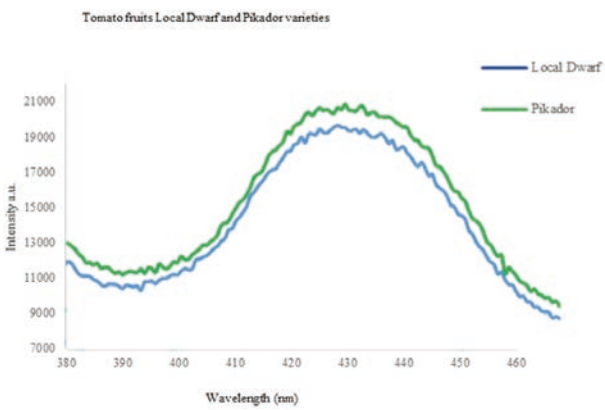

Figure 2: Emission wavelengths of tomato fruits Local Dwarf and Pikador varieties

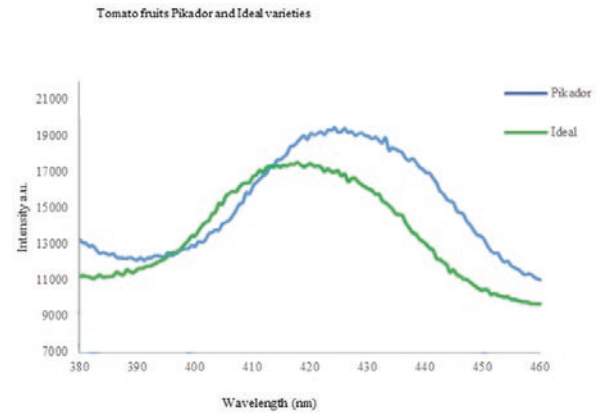

Figure 4: Emission wavelengths of tomato fruits Pikador and Ideal varieties

fruit tomatoes from different varieties. Fluorescence spectroscopy can be applied to analyze the tomato fruit of unknown cultivars and establish its origin with a sufficiently well-structured data library. Because it can be applied topically to test specimens. It eliminates sample damage during transport and provides a highly sensitive assay.

4 CONCLUSIONS

The fluorescence spectroscopy method is fast-acting in application as a field method in the determination of varietal differences after tomatoes harvesting locally under uncontrolled conditions.

It has been proven that fluorescence spectroscopy will successfully apply as a rapid tool to establish the origin of unknown tomato fruits in the presence of a rich library of spectra. The developed method can be used successfully in tomato breeding programs. The stability of the breeding line and its common blacks with an established cultivar of the same species can be observed by following the difference in the spectral distribution.

The differentiation of related varieties is a laborious and time-consuming task. For these reasons, the development of techniques that *could* assist in an early, quick, and accurate differentiation of related varieties is of utmost importance.

A systematic engineering approach to the setup (optical setup) of a mobile fiber optic plant for fluorescence spectroscopy research was found to be applicable in determining varietal differences in tomato cultivation.

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