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Application of fluorescence spectroscopy as a field method in the determination of varietal differences radish (*Raphanus sativus* L.var. *sativus*) accessions after harvesting

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Application of fluorescence spectroscopy as a field method in the determination of varietal differences radish (*Raphanus sativus* L.var. *sativus*) **accessions after harvesting**

Abstract: The aim of the study is to establish the application of fluorescence spectroscopy as a field method for determining post-harvest varietal differences in radish accessions (*Raphanus sativus* L.var. *sativus*). The proposed method includes studies of radish root crops from standard varieties and those from first-generation hybrids by means of fluorescence spectra. They will be compared in terms of determining the spectral distribution due to the varietal differences of a given genotype. Specimens were grown under uncontrolled field conditions. This will allow the approach to be practiced non-invasively in the quality control of radish production on undefined premises and outdoors. The experimental studies were carried out locally on the farm. The spectral installation for generating emission fluorescence spectra is mobile. In its setup (optical setup), 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 radish genotypes after harvest under uncontrolled conditions. The stability of the breeding variety and its similarities with an established variety of the same species can be observed by monitoring the intensity of the signal. This will assist the process of determining the belonging of a studied plant to a certain variety or breeding line, even for samples of unknown origin, when it is necessary to qualify the result of the samples in a short time.

Key words: radish accessions (*Raphanus sativus* L.var. *sativus*), varietal differences, uncontrolled conditions; field method; fluorescence spectroscopy

Uporaba fluorescenčne spektroskopije kot poljske metode za določanje sortnih razlik akcesij redkvice (*Raphanus sativus* **L. var.** *sativus*) **po spravilu**

Izvleček: Namen raziskave je bil vzpostaviti uporabo fluorescenčne spektroskopije kot poljske metode za določanje sortnih razlik akcesij redkvice (*Raphanus sativus* L. var. sativus) po spravilu. Predlagana metoda obsega preučevanje standardnih sort redkvice in križancev prve generacije na osnovi fluorescenčnih spektrov, ki bodo primerjani glede na razlike med sortami znotraj določenega genotipa. Preiskušane rastline so bile gojene na prostem v kontroliranih razmerah. Metoda bo omogočala neinvazivno kontrolo kakovosti v pridelavi redkvice, pridelane tudi v neznanih okoljih na prostem. Poskusi so bili izvedeni na posameznih kmetijah. Naprava za generiranje emisijskih fluorescenčnih spektrov je mobilna. Pri njeni postavitivi je bil uporabljen klasični inženirski pristop za postavitev modernih optično-elektronskih naprav. Rezultati raziskave bodo lahko uporabljeni za optimizacijo časa pri analizah sortnih razlik genotipov redkvice po spravilu v nenadzorovanih razmerah. Stabilnost gojenih sort in njihova odstopanja bodo določena na osnovi jakosti signala uveljavljene sorte. To bo pripomoglo pri določitvi, če določena preučevana rastlina pripada določeni sorti ali le gojitveni liniji, celo za vzorce neznanega izvora, še posebej, ko je potrebno hitro opredeliti kakovost vzorcev.

Ključne besede: akcesije redkvice (*Raphanus sativus* L. var. *sativus*), sortne razlike, nenadzorovane razmere, poljska metoda, fluorescenčna spektroskopija

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1 INTRODUCTION

Radish (*Raphanus sativus* L.var. *sativus*) is an annual root vegetable plant from the Cruciferous family. It originates in Central Asia (Kaneko et al., 2007). And has been cultivated as a vegetable crop since about 1000 BC in China, Japan, Egypt, Rome, and Greece (Perez Gutierrez et al., 2004). There are two groups of varieties: European and Chinese.

Radishes are for fresh consumption (Kyung-Mi et al., 2015).Their widest application is in making fresh vegetable salads. They are not suitable for heat treatment. Radishes are easy-to-grow root vegetables. They tolerate most soil types and grow rapidly (Hyde et al., 2012).

Optoelectronic methods, including fluorescence spectroscopy, are well-established techniques for the determination of chemical components in foods (Butzet al., 2005; Nicolaï et al., 2007). The advantages of optoelectronic methods include the rapidity of the analysis, the low cost of the test, and the possibility of simultaneous evaluation of several markers from one spectrum (Dakin and Brown, 2006; Mitchke, 2010). These advantages make them suitable for application in breeding programs and quality assessment when many samples need to be analyzed.

Optoelectronic methods are also used as rapid and non-destructive techniques to measure the intrinsic quality of various biological objects. They are successful techniques in the determination of dry matter and soluble solids content (SSC) of fruits and vegetables due to the absorption of sugar and water. Studies are available on the determination of SSC in apples (Lu et al., 2011) and tomatoes (Ecarnot et al., 2013).

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 for instant analysis of warehouse production of radish in uncontrolled conditions, both in processing plants and in food retail outlets.

The present study aims to establish the feasibility of fluorescence spectroscopy as a field method in the determination of varietal differences after radish harvesting. The differential parameter, utilized in our study is the spectral distribution due to the varietal differences of a particular genotype. The specimens were grown under uncontrolled field conditions. This will allow the application of the technique to the non-invasive quality control of radish production in unspecified rooms and outdoors.

2 MATERIALS AND METHODS

2.1 MATERIAL

Accessions of three standard radish varieties and one first generation hybrid variety were investigated:

- ‘French Breakfast’: The variety is suitable for spring and autumn field production. The hypocotyl tubers are single and oblong, with a white five around the tail. The fleshy part is white and crispy. The vegetation period is 30 days. The sowing rate is 1.5-2.5 kg per hectare
- ‘Nacional 2’: The variety is an early field variety that produces large, round, red roots with white tips. Its flesh is white, delicate in taste and crunchy.
- Red large: The variety is medium early. It is suitable for spring and autumn field production. The fruits are single, deep red, the fleshy part is white with excellent taste qualities. The variety is resistant to cracking.
- ‘Espresso F1’: A very early variety with round root tubers colored red. It is hardy with a fine root, a strong bond with the foliage. It has very good transportability and storage. It is recommended for growing in winter and very early in open areas

Radishes are grown according to standard technology. The seeds are sown in evenly spaced rows on an area of 1.6 m^2. The distance between the rows is 8 cm and the depth of the furrow is 2 cm. The seeds should be 3 cm apart in the row itself. The seeds are sown in moist soil.

2.2 FLUORESCENCE SPECTROSCOPY

The mobile fiber-optical spectral installation for the study of fluorescence signals is designed specifically for

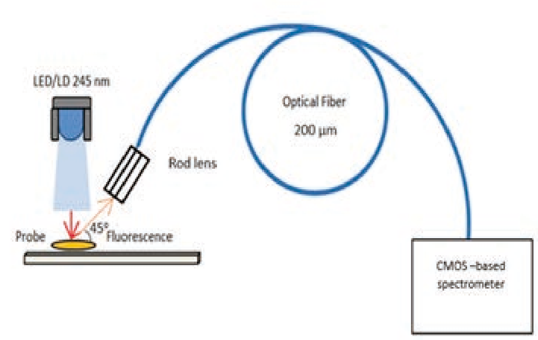

Figure 1: Mobile experimental installation used by fluorescence spectroscopy .

the rapid analysis of plant biological samples. The mobile experimental setup used by fluorescence spectroscopy includes the following components:

- Laser diode (LED) with an emission radiation of 245 nm with a supply voltage in the range of 3V. It is housed in a hermetically sealed TO39 metal housing. The emitter has a voltage drop from 1.9 to 2.4 V and a current consumption of 0.02 A. The minimum value of its reverse voltage is – 6 V.
- Rod lens of the achromatic doublet type. It is composed of two bonded lenses with different Schott and Corning dispersion coefficients with an anti-reflective coating. The radii of the two lenses are selected so that the chromatic aberration of one lens compensates that of the other. The tolerance of the diameter of the forming optics is -0.005 mm.
- The multimode optical fiber is FG200LEA. It has a core diameter of 200 µm and a step index of refraction.
- Quartz plate: area 4 cm^2. Its optical properties are to be transparent to visible light and to ultraviolet and infrared rays. This allows it to be free of inhomogeneities that scatter light. Its optical and thermal properties exceed those of other types of glass due to its purity. Light absorption in quartz glasses is weak.
- CMOS detector with photosensitive area 1.9968 × 1.9968 mm. Its sensitivity ranges from 200 nm to 1100 nm. Its spectral resolution is $\delta\lambda = 5$. The profile of the detector sensor projections along the X and Y axes is also designed for very small amounts of data, unlike widely used sensors.

The sample is irradiated by the LED, after which it fluoresces. The emission signal is received at 45 ° by the rod lens, which transmits it through the optical fiber to the detector. The three unique advantages of this scheme are:

- Inclusion of the rod lens in the construction of the system. Due to its increased light transmission efficiency by almost completely filling the air gaps between the individual lenses.
- Unique design of optical fiber coupling from a headquarters lens in a duralumin housing. In this way, the most optimal for compiling with optical fibers and forming images from laser diodes with low levels of intense losses is achieved.
- The sample fluoresces after being irradiated by the LED. The emission signal is received at 45 ° from Rod Len, and the emission signal is generated. It is then transmitted through the optical fibre to the detector.

3 RESULTS AND DISCUSSION

Radishes contain specific fluorophores/chromophores/ scatterers. The most important of these are myrosinase, glucosinolate and isothiocyanate. They determine their spectral properties.The possible transitions between these energy levels, as a function of photon energy, are specific to radishes, resulting in spectra and

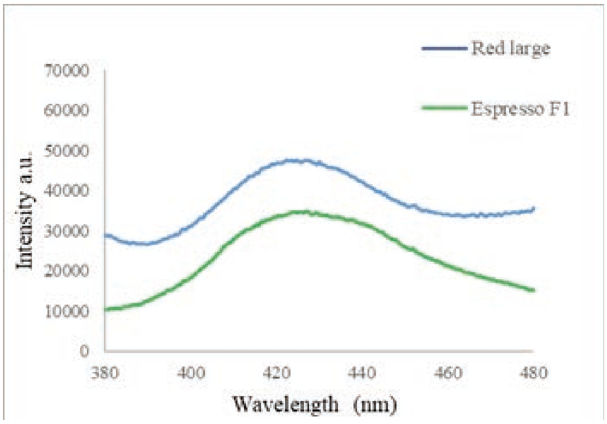

Figure 2: Emission wavelengths of 'Red Large' and 'Espresso F1'

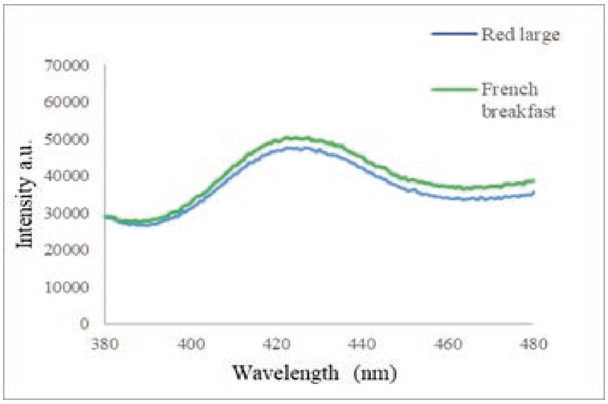

Figure 3: Emission wavelengths of 'Red Large' and 'French Breakfast'

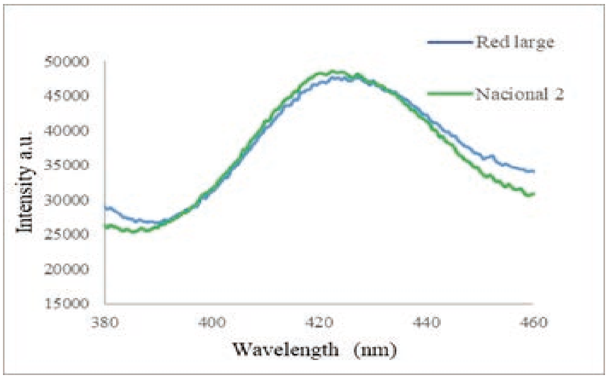

Figure 4: Emission wavelengths of Red Large and Nacional 2..

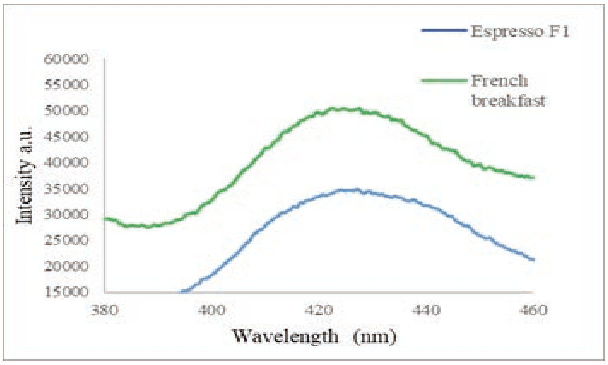

Figure 5: Emission wavelengths of 'Espresso F1' and 'French Breakfast'.

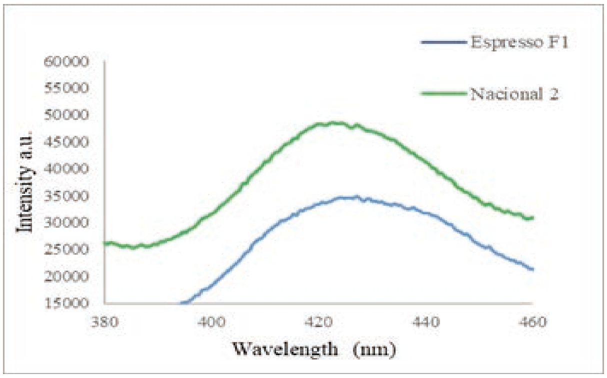

Figure 6: Emission wavelengths of 'Espresso F1' and 'Nacional 2'.

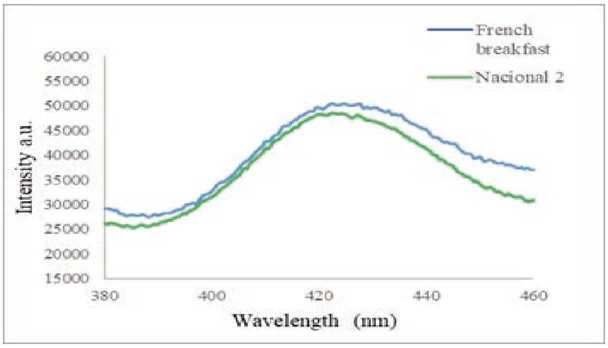

Figure 7 Emission wavelengths of 'French Breakfast' and 'Nacional 2'.

optical properties unique to them. Radishes contain structures smaller than the wavelength of visible light. When specific fluorophores are irradiated in the radishes, light with a wavelength also greater than that of the exciting radiation is emitted, but with this difference that the afterglow with is too short-lived. In this process, an electron absorbs a high-frequency photon, makes at least one emissionless transition to a lower energy level, then undergoes a relaxation process to its ground level and re-emits a low-frequency photon Radishes contain non-uniform particles smaller than the wavelength of visible light, and their orientation also affects the degree of polarization. In turbid media such as radish fluorophore compounds act as independent light sources, emitting incoherently, causing their visible fluorescence. The presence of fluorophores (myrosinase, glucosinolate and isothiocyanate) in radishes allows their qualification by fluorescence spectroscopy. Therefore, fluorescence spectroscopy finds application for analysis of this vegetable crop.

The optical parameters and spectral properties also change as a function of temperature, pressure, external electric and magnetic fields, etc., which allows obtaining essential information about changes in the chemical and cellular morphological composition of radishes. This gives us reason to claim that, for the first time, to the best of our knowledge, fluorescence spectroscopy has been applied to analyze radishes regarding their varietal differences under uncontrolled conditions, to the best of our knowledge. A difference in the emission fluorescence signal of the different varieties was clearly observed.

The results give reason to conclude that fluorescence spectroscopy can be successfully applied as a rapid tool to establish the origin of unknown root radishes in the presence of a rich library of spectra. This will be an applied tool in selection programs. For processing from the fluorescence study, 10 averaged plots from 4 different radish cultivars are presented. Graphs are averaged after the 15th measurement of each sample. Emission spectral data are decomposed into relations in such a way that only primary facts are stored in each relation. In this way, the truth of the real sample plot is preserved by tracking signal intensity, one can monitor the stability of a cultivar and its common blacks with other varieties. The emission fluorescence signals of 'French Breakfast' and 'Nacional 2' (Figure 7), 'Red Large' and 'Nacional 2' (Figure 4), and 'Red Large' and 'French Breakfast' (Figure 3) are close in terms of wavelength localization and signal intensity level. This is expected from the fact that the varieties have a similar cell-morphological composition when grown in open air. The emission fluorescence signals of radish root tubers were analyzed. As they are located at 45 degrees to the receiving multimode fiber. Radishes are placed on a quartz substrate, with their root part oriented downwards. Radishes are available with their root part below 45 degrees. This placement was chosen because it was found that the most informative emission fluorescence signals were obtained at this position of the irradiating signal. Experiments were conducted for the most informative placement of the radish tubers, in order to choose the most optimal placement However, the method of fluorescence spectroscopy can be applied to distinguish the root tubers of these two varieties because the correlation in the spectral distribution is sufficiently distinct and distinguishable to determine practically qualitatively the belonging of the roots to a given variety. The method of fluorescence spectroscopy can practically be used to qualitatively determine the belonging of a root radish to a given variety.

It turned out that we couldn't find any previous applications of the described experimental approach for the field method in the determination of varietal differences after radish harvesting has not been applied internationally. This gives us reason to claim that for the first time, fluorescence spectroscopy was applied to the application of fluorescence spectroscopy as a field method in the determination of varietal differences after radish harvesting under uncontrolled conditions. The method is successfully applied to distinguish root radishes from different varieties. Fluorescence spectroscopy can be applied to analyze radish root crops 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 radish 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 radish accessions in the presence of a rich library of spectra. This will be an applied tool in selection programs. By monitoring the signal intensity, the stability of a breeding line and its common blacks with an established variety of the same species can be monitored.

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

It has been established that the system engineering approach for adjustment (optical adjustment) of a specialized installation for applied research with fluorescence spectroscopy is applicable in the determination of varietal differences during radish breeding.

5 ACKNOWLEDGMENTS

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