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

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Abstract: The present study aims to establish the application of fluorescence spectroscopy as a field method in the determination of varietal differences after black radish harvesting. The experimental studies were conducted on site at the farm where the black radish accessions were grown. The fluorescence analysis was carried out with a source with an emission wavelength of 285 nm and an author-developed mobile fiber-optic experimental set-up. The subjects of this research are root crops from Black Spanish round, Black Spanish long, Rattail, and Nero Tondo. The correlation between the emission signals of the samples was established. This fact allows mobile fluorescence spectroscopy to be successfully applied as a rapid tool to establish the origin of unknown black radish root crops in the presence of a rich library of spectra as an applied tool in breeding programs. The results of the experiment can be used to optimize the time for the analysis of the varietal differences of the black radish genotypes after harvest. Fluorescence spectroscopy in a fiber-optical configuration will support the process of determining the belonging of a specific variety to a given variety, even for samples of unknown origin, when it is necessary to qualify the result of accession in a short time.

Key words: black radish accessions; fluorescence spectroscopy; variety; emission wavelength

INTRODUCTION

Black radish (*Raphanus sativus* L. var. niger) is an annual or biennial vegetable plant, a root crop. It is classified under the Brassicaceae family (Yücetepe et al., 2021).

The cultivation of the black turnip dates back to ancient Egypt. The region of present-day Syria is considered the geographical origin of the black turnip. It was first mentioned in Europe in 1548. In the 16th century, it was one of the most common types of turnips on the old continent (Singh, 2021). The juice of the fresh root of black radish is used for medical purposes. For storage, mature plants can be kept in the ground if temperatures are low (Jeon et al., 2022). Black radishes can also be stored for months in a root cellar or in the refrigerator for up to three weeks at 0–5 °C (Barimani et al., 2020).

The approach based on chlorophyll-a fluorescence transient analysis is known as the JIP-assay (Strasser et al., 2004). The JIP-assay is one of the most popular tools in photosynthetic studies (Kalaji et al., 2014; Chen et al., 2014; Zhang et al., 2016). Illumination of dark-adapted leaf tissue results in rapid chlorophyll (PF) fluorescence, whose induction curves are called O-J-I-P. The resulting curves provide information on the functioning and structure of the photosynthetic apparatus. The characteristic name of the OJIP test derives from the specific points of the chlorophyll fluorescence signal curves (Tsimilli-Michael et al., 2013). Chlorophyll fluorescence induction curves are based on the theory of energy flow in thylakoid membranes. The parameters of the JIP-test are categorized into four groups: data extracted from the recorded fluorescence transients, quantum yields and probabilities, vitality indices, and specific energy fluxes, which are divided into phenomenological and specific categories (Strasser et al., 2000). The kinetics of the JIP assay can be a useful indicator of the negative environmental impact on photosynthetic organisms, including nutrient deficiency (Kalaji et al., 2014, 2016). By applying a mobile fiber-optic setup configuration using the phenomenon of the fluorescence of light, it is possible to create non-invasive methods for the evaluation of varietal differences in black radish. Until now, there has been no data on their characterization using the proposed method.

The aim is to validate fluorescence spectroscopy in the proposed configuration as a non-invasive method for determining the varietal differences of black radish accessions after harvesting.

As a result of the successfully applied research, it is expected that the non-invasive method will be used to optimize the time for the analysis of the varietal difference of black radish genotypes after harvest, under uncontrolled conditions.

MATERIAL AND METHODS

Material:

Accessions from four standard carrot varieties were investigated:

Black Spanish Round: Root crops are globose, up to 2-1/2 inches in diameter, with crisp, hot white flesh that holds well after picking. The variety can be sown in early spring, but the harvest is quiet in winter if sown in July–August.

Black Spanish Long: A large root vegetable that is dark brown with long pointed roots but white flesh. It can be left in the ground and harvested in winter or stored in dry sand in a frost-free shed. It is believed to aid digestion and is high in antioxidants.

Rattail: Root crops are flat-oval to oblong, with an intensively obtuse eye on the surface and a diameter of 10–12 cm. The top of the open-faced papaya copta is a white, dense, and pungent meat. This variety is suitable for growing in winter

Nero Tondo: This elite variety is much more uniform and resistant to curling than the common black Spanish variety. Large 2–4" (depending on harvest date) black, round roots with crisp, hot, white flesh. Suitable for mid-spring sowing (soil temperature 60°F/16°C or higher, to prevent warping) until autumn, medium long storage.

The black radish accessions are grown according to standard technology. Beds are formed in which rows are formed. In these rows, black turnip seeds are planted in nests (3-4 per nest). Planting was done according to a 20×40 scheme, where 20 cm is the distance between plants and 40 cm is the distance between rows. The seeds are planted in moist soil at a depth of 2-3 cm, covered with earth, slightly compacted. The agrotechnical measures were carried out in the optimal terms for the culture.

Fluorescence spectroscopy:

Accessions of four different varieties black radish after harvesting were subjected to fluorescence spectroscopic measurements. The mobile spectral installation (Figure 1) for the study of fluorescence signals was designed specifically for the rapid analysis of plant biological samples.

The mobile experimental installation used by fluorescence spectroscopy contains the following blocks:

• 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 of 1.9 to 2.4V and a current consumption of 0.02A. The minimum value of their reverse voltage is – 6 V.

• Forming optic, which is a hemispherical lens made of N-BAK2 glass. The post-LED forming optics is defined mainly for its refractive, dispersive and thermo-optical properties, as well as for its transparency in the UV range [240-280 nm].

• Quartz glass area 4 cm2. Its optical properties are to be transparent to visible light and to ultraviolet rays. This allows it to be free of in

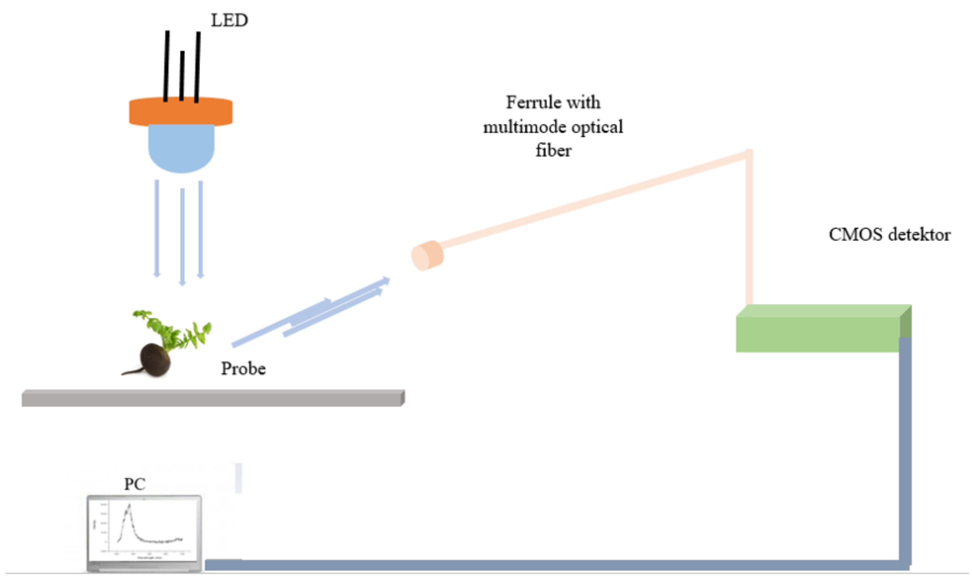

Figure 1. Mobile experimental installation used by fluorescence spectroscopy

homogeneities 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 resolution is δλ= 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 obtained fluorescence spectroscopic data subjected to statistical analysis to distinguish 4 different varieties of black radish after harvesting.

RESULTS AND DISCUSSION

For the particular circuit, the photo detector is of the CMOS model AR0521SR type. Its sensitivity is in the range of 200 nm to 1200 nm. Its resolution is δλ= 3 nm. The AR0521SR was chosen because it can detect very high intensity root emission radiation.

The sample is irradiated by the source. After the sample fluoresces and the emission signal falls on the 45o fiber, which carries the signal to the detector.

The optical properties of a black radish are determined by its energy structure, which includes both the occupied and free electronic energy levels, as well as the energy levels of the atomic vibrations of the molecules or the crystal lattice. The possible transitions between these energy levels, as a function of photon energy, are specific to black radish, resulting in spectra and optical properties unique to it. Black radish contains particles smaller than the wavelength of visible light. Particles in the turbid medium, such as black radish, act as independent light sources, emitting incoherently, causing the samples to visibly fluoresce.

Therefore, fluorescence spectroscopy finds application for analysis in this culture. 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 black radish. This gives us reason to claim that, for the first time, fluorescence spectroscopy has been applied to the analysis of black radish samples regarding their varietal affiliation under uncontrolled conditions. A difference in the emission fluorescence signal of black radish accessions from different varieties is clearly observed.

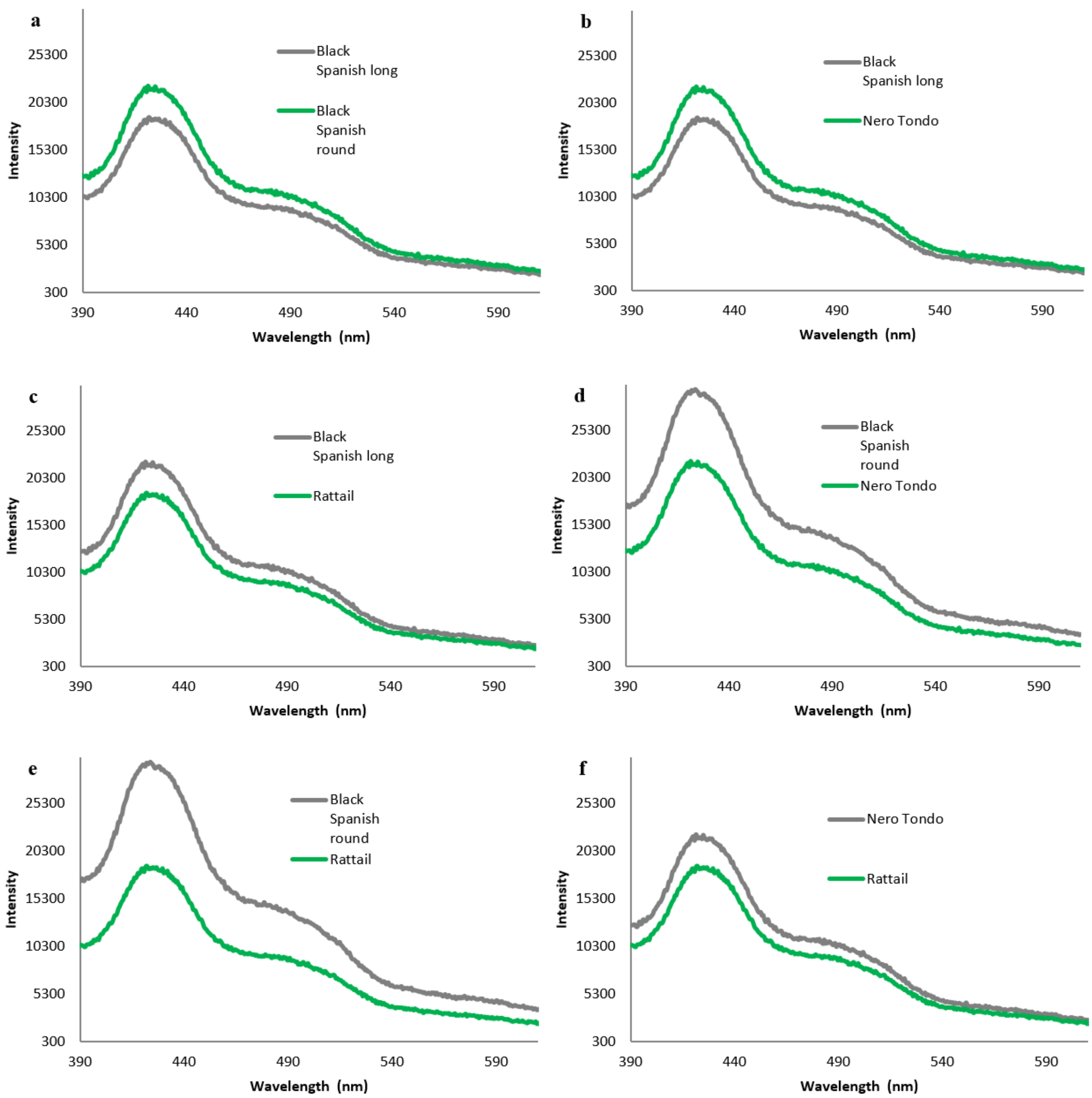

Figure 2. Difference in emission wavelength for Black Spanish round, Black Spanish long, Rattail and Nero Tondo

Table 1. Numerical differences in emission wavelength and Intensity for Black Spanish round, Black Spanish long, Rattail and Nero Tondo

Variety	1 Emission wavelength (nm)	Intensity (a. u.)	2 Emission wavelength (nm)	Intensity (a. u.)
Black Spanish long	427	18588	499	8848
Black Spanish round	424	29432	486	13405
Nero Tondo	421	21773	481	10384
Rattail	398	18236	494	7855

The results give reason to conclude that fluorescence spectroscopy can be successfully applied as a rapid tool to establish the origin of unknown black radish root crops in the presence of a rich library of spectra. This will be an applied tool in breeding programs. Figure 2 shows the spectral distributions of Black Spanish round, Black Spanish long, Rattail and Nero Tondo accessions. For a better visualization, since emission wavelengths are difficult to distinguish graphically, table 1 is presented with the numerical values of intensity and emission wavelengths By tracking signal intensity, one can monitor the stability of a variety and its general characteristics with other varieties. Emission fluorescence signals of Black Spanish long, Rattail and Nero Tondo (Figure 2 b) and care close in terms of wavelength localization and signal intensity level. This is expected from the fact that the cultivars have a similar cell morphological composition when grown outdoors. However, the method of fluorescence spectroscopy can be applied to distinguish the root crops of these three varieties, since the correlation in the spectral distribution is sufficiently distinct and distinguishable to determine practically qualitatively the belonging of the root crop to a given variety. The method of fluorescence spectroscopy can practically be used to qualitatively determine the belonging of a black radish root crop to a given variety.

A literature review aimed at conducting such research was conducted. It turned out that the experimental approach described so far for the Polish method for determining varietal differences after harvest of black radish has not been applied internationally. This gives us reason to claim that, for the first time, fluorescence spectroscopy has been applied as a field method in the determination of varietal differences after harvesting black radish under uncontrolled conditions. The method has been successfully applied to distinguish black radish root crops from different varieties. Fluorescence spectroscopy can be applied to analyze black radish root crops of unknown varieties and establish their origin with a sufficiently well-structured data library. Because it can be applied topically on trial samples. The application of the mobile circuit eliminates sample damage during transport and provides highly sensitive analysis.

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CONCLUSION

- The fluorescence spectroscopy method is fast-acting in application as a field method in the determination of varietal differences after black radish harvesting locally under uncontrolled conditions.
- It has been proven that fluorescence spectroscopy will help apply successfully as a rapid tool to establish the origin of unknown black radish root crops in the presence of a rich library of spectra. This will be an applied tool in breeding 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 an early, quick and accurate differentiation of related black radish varieties is of the 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 black radish breeding.

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