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Original article

Application of Mobile Fluorescence Spectroscopy as a Method in the Determination of Varietal Differences in Parsley (*Petroselinum Crispum*) Seeds

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Abstract

Standard methods used for seed quality are relatively slow and require expensive supplies. An optical mobile installation for the study of parsley seeds (*Petroselinum crispum*) has been successfully set up and tested. The present study aims to establish the application of mobile fluorescence spectroscopy as a method to determine varietal differences in parsley seeds. The proposed method includes the examination of parsley seeds of different varieties with a mobile fiber optic system by means of fluorescence spectroscopy. Spectral distributions are unique to seeds of a particular variety. This fact justifies the use of the plant to recognize available parsley seeds of unknown origin in a non-invasive way with high accuracy. The stability of the breeding line and its common blacks with an established variety of the same species can be monitored by monitoring the signal intensity. The stability and signal intensity level of is close. The spectral distribution with wavelengths of the reflected emission of the studied parsley seeds reflects the characteristic distribution of the standard varieties. The installation can be applied with high accuracy to study parsley seeds in the field.

Keywords: Mobile spectral installation, Fluorescence spectroscopy, Parsley seeds, Different varieties.

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INTRODUCTION

Parsley (Petroselinum crispum) is a biennial herb and spice widely distributed in the Middle East, Europe, North and South America (Sarwar et al.,2016).

The two most popular types of parsley are curly parsley and Italian flat-leaf parsley. The Italian variety is more aromatic and less bitter in taste than the curly one. There is another type of parsley known as root turnip (or the Hamburg variety) that is grown for its burdock-like roots. Parsley belongs to the umbel family. People began consuming parsley as a spice sometime during the Middle Ages in Europe (Sun et al., 2016).

In some countries the curly variety is more popular. This stems from ancient preferences for this species, as people likened plantain parsley to a type of poisonous grass (Punosevac et al., 2021).

The development of fast and accurate methods such as optical diagnostics based on non-destructive analysis will help to overcome the barriers in studying and mon-itoring the processes related to seed germination and guarantee quality planting mate-rial and seeds for agro-industry and farmers (Singh et al., 2021) .

The optoelectronic methods for assessing the quality of plant seeds are non-contact, fast-acting, selective, and do not destroy the integrity of the examined sample. On the basis of these, it is possible to create non-invasive methods for the evaluation of parsley seeds. Until now, there has been no data on their re-search using the proposed method. Obtained results in the study of cereal seeds (Boothe et al., 2010). Based on his research, emission excitation wavelengths of 362 nm , 424 nm and 485 nm were established. In these studies, it was found that during the ripening of seeds of cereal plants (for example, wheat, oats, and corn), the ratio of their excitation levels and changes in radiation for immature seeds is characteristic of the short-wave range, and long-wave prevails in mature seeds (Belyakov et al., 2017). The dependence of the ratio of long-and short-wavelength fluxes on the maturation time increases and can be statistically reli-ably approximated by the linear functions required to create a database (Vidal-Valverde and Frias, 2010).

Belyakov et all (2021) developed a sensor for determining the level of physiological maturity of seeds, allowing by irradiating seeds with two sources at certain wavelengths and re-cording the photoluminescent flow with appropriate receivers to determine the stage of seed maturation. The maximum luminescence is less pronounced than in the exci-tation spectrum.

Measured spectral luminescence characteristics of forage plant seeds were measured by scarification during the study. The spectral characteristics of the seeds are in-crease, due to the scarification of forage plants (Huyan et al., 2018). It was established that in the studied seeds with repeated scarification, the observed qualitative changes in the excitation spectrum were related to the appearance of a new maximum at a wavelength of 423 nm. Likewise, for parsley seeds from four standard varieties, the obtained results can be used to create a schematic fiber-optic configuration for character-ization of planting material from parsley (Quan et al., 2019).

The excitation and photoluminescence spectra of seeds of agricultural plants, legumes and tomatoes were measured using a previously developed method. The typical excitation spectrum was found to be in the range of 355–500 nm and to have two maxima: the main one at 424 nm and the side one at 485 nm. The emission spectrum is in the range of 420–650 nm and has a maximum in the region of 500-520 nm (Belyakov et al., 2019).

The water contained in the planting material (together with impurities) is expressed as a percentage of its total mass. In addition, it will be possible to evaluate the percentage of their normal seed germination under optimal conditions for germination in a period shorter than the period for which the germination rate is determined, which will determine their germination energy (Bouasla et al., 2022).

The present study aims to establish the function of fluorescence spectroscopy as a mobile method for the analysis of representatives of different varieties of parsley seeds . The application of optoelectronics in the analysis of parsley seeds will lead to a rapid and correct determination of the sowing rate, since it will be possible to assess the germination capacity of all clean seeds. A study of parsley seeds from standard varieties was conducted, which aims to develop a non-invasive method for their quality, through the application of the system engineering approaches of modern optoelectronics.

MATERIALS and METHODS

Material

Seeds of four standard parsley varieties and one first generation hybrid variety were investigated:

- Italian flat-leaf parsley – It is an early very high-yielding variety distinguished by extremely large and leafy rosettes that reach a height of 69-90 cm. The leaves are highly aromatic, broad, glossy and of a rich dark green color. The variety is very adaptable, does not form root crops and is preferred both for fresh consumption and as a dried spice. Vegetation period 75-85 days.
- Curly parsley - It is an early and productive variety. The leaves are strongly curled, tender and fragrant. Medium green. It is suitable for decorating salads and dishes. Grows best in full sun. Curly parsley is sown directly and you do not need to prepare seedlings. Spring sowing of the seeds takes place between February 20 and March 10. Do the pre-winter sowing of the seed in the period between December 1 and 15. The vegetation period lasts about 70-80 days. It is grown for its highly aromatic curly leaves, which are rich in essential oils and numerous vitamins.

- Einfache Schnitt - Smooth-leaved, very aromatic variety with a high yield. With late sowing, a harvest is possible in autumn until the following spring. Suitable for beds, containers and balcony boxes. Make sure the soil is loose and avoid waterlogging. Parsley loves a moist, semi-shady, humus-rich and nutritious location.

Komon 2 - This variety is a standard selection for market and home gardens. It produces flat, smooth, deeply indented, dark glossy green, medium-sized leaves with a strong aroma. Parsley grows best in a mostly sunny location with relatively rich, moist and well-drained soil.

Fluorescence spectroscopy

The mobile fiber-optical spectral installation for the study of fluorescence signals is designed specifically for 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 volt-age in the range of 3V. Its input power is 16 mW. 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

- 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 glass area 4 cm2. 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 resolution is $\delta\lambda = 5$. The profile of the detector sen-sor 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^o 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 their 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.
- Reception of the emission signal at 45o

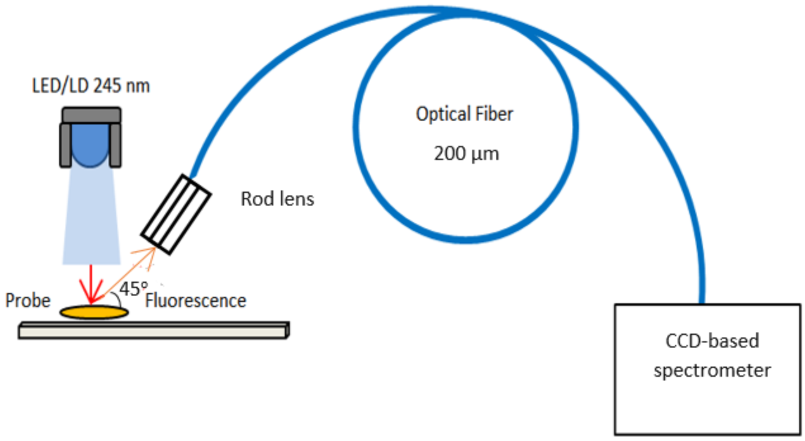

Figure 1. Mobile experimental installation used by fluorescence spectroscopy

Study Indicators:

Spectral analysis - a spectral analysis of 10 tubers of each variety and each breeding line was carried out. The emission spectrum represents the wavelength distribution of an emission measured for a constant excitation wavelength. The excitation spectrum represents the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength. This spectrum is represented as a function of the wavelength of the light intensity incident on the photodetector in the spectrometer.

Spectral analysis indicators are:

- Exciting wavelength
- Emission wavelength
- Intensity strength

RESULTS and DISCUSSION

During the tests carried out by means of optoelectronic spectroscopy of the seeds, by means of a fiber-optic spectrometer, a clear correlation between their emission signals is visible. This study shows that fluorescence spectroscopy is applicable in the study of parsley seeds. The spectral setup based on fluorescence signals allows recording both the emission spectrum and the spectrum of the excitation source. The emission spectrum represents the wavelength distribution of an emission measured for a constant excita-tion wavelength. The excitation spectrum represents the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength. This spectrum is represented as a dependence of the light wavelength on the light in-tensity falling on the photodetector, which is part of the installation.

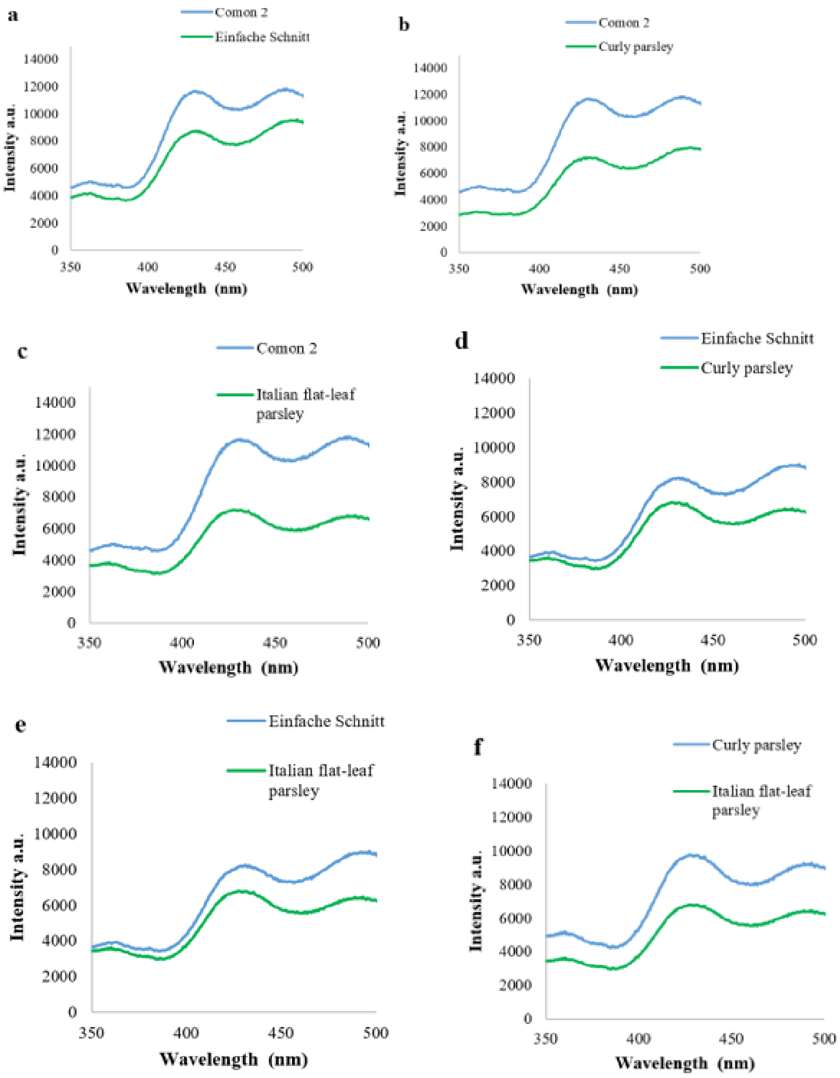

Figure 2. Difference in emission wavelength for seeds of Einfache Schnitt, Italian flat-leaf parsley, Komon 2 and Curly parsley

The three main advantages of fluorescence spectroscopy are that the method is fast, does not require consumables, and can be performed in situ in the warehouse under uncontrolled conditions. The decision for local measurements was made to avoid damage to the samples during transport and thus, to ensure fluorescence analysis with high sensitivity.

The optical properties of parsley seeds 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 parsley seeds , resulting in spectra and optical properties unique to it. Parsley seeds contain particles smaller than the wavelength of visible light. Particles in the turbid medium, such as parsley, act as independent light sources, emitting incoherently, causing the samples to visibly fluoresce.

Therefore, fluorescence spectroscopy finds applications for analysis in these seeds. 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 parsley seeds. This gives us reason to claim that, for the first time, mobile fluorescence spectroscopy has been applied to the analysis of parsley seeds regarding their varietal affiliation under uncontrolled conditions. A difference in the emission fluorescence signal of parsley seeds accessions from different varieties is 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 parsley seeds in the presence of a rich library of spectra. This will be an applied tool in breeding programs. By tracking signal intensity, one can monitor the stability of a variety and its general characteristics with other varieties. Emission fluorescence signals of Italian flat-leaf parsley, Komon 2 and Curly parsley (Figure 2 d, e and f are 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 seeds of these three varietes, since the correlation in the spectral distribution is sufficiently distinct and distinguishable to determine practically qualitatively the belonging of the seeds to a given cultivar. The method of fluorescence spectroscopy can practically be used to qualitatively determine the belonging of a parsley seed to a given variety.

Conclusion

The systems engineering approach of adjustment (optical setting up) a specialized flu-orescence spectroscopy applied research setup was found to be applicable in the anal-ysis of parsley seeds from standard varieties and those from first generation hybrids.

A non-destructive method for evaluating pretreated parsley seeds from standard varie-ties and those from first generation hybrids are demonstrated.

With a sufficiently well-structured data library, fluorescence spectroscopy can be ap-plied to analyze parsley seeds from standard varieties.

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