



Original article

## Application of Mobile Fluorescence Spectroscopy as a Method in the Determination of Varietal Differences in Lettuce (*Lactuca Sativa*) after Harvesting in Greenhouse Cultivation

Vanya Slavova \*

Department of Plant Breeding, Maritsa Vegetable Crops Research Institute, Agricultural Academy of Bulgaria, Bulgaria

### Abstract

The present study aims to establish the application of fluorescence spectroscopy as a field method in the determination of varietal differences after lettuce harvesting in greenhouse cultivation. The experimental studies were conducted on-site at the farm where the lettuce leaf 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 leaves from Djentelina, Hercules, Lolo Roca, and Paris White.

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 lettuce accessions 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 lettuce genotypes after harvest in greenhouse cultivation. Fluorescence spectroscopy in a fiber-optical configuration will support the process of determining the belonging of a specific variety of lettuce to a given variety of lettuce (even for samples of unknown origin when it is necessary to qualify the result of accessions in a short time).

**Keywords:** Mobile fluorescence spectroscopy, Lettuce accessions, Varietal differences.

**Received:** 30 April 2024 \* **Accepted:** 02 June 2024 \* **DOI:** <https://doi.org/10.29329/ijjaar.2024.1049.7>

---

\* **Corresponding author:**

Vanya Slavova, Department of Plant Breeding, Maritsa Vegetable Crops Research Institute, Agricultural Academy of Bulgaria, Bulgaria.  
Email: [vania\\_plachkova@abv.bg](mailto:vania_plachkova@abv.bg)

## INTRODUCTION

Lettuce (*Lactuca sativa*), or garden lettuce, is an annual plant. There are three varieties in total: cephalic, leafy, and romen. Lettuce is grown in a 3–4 year crop rotation with a mandatory two-year interval between growing seasons due to the accumulation of soil infections (Mou, 2009). Lettuce is best planted in areas where grains, potatoes, common cabbage, garden leeks, or celery grow (Mou, 2008).

Lettuce, as a cold-resistant plant, is most often planted in spring or autumn, with the possibility of planting in winter. For its successful cultivation, it is important that the planting take place on well-treated and rich organic matter soil (Worth et al., 2023). Preliminary land preparation, which includes deworming, is key to successful plant development. (Panwar et al., 2021). The lower temperatures in autumn are good for the taste qualities of this crop. Also, as frost approaches, there is less danger from bug attacks. (Tudela et al., 2017). Although vegetable consumption is lower in the winter, it is beneficial to have fresh lettuce leaves available. It is quite possible to grow lettuce in the winter with little effort (Beattie, 1940).

Lettuce grown in autumn and winter is divided into two types. Lettuce sown in late summer will withstand light frosts that may occur in October and November. Lettuce is harder than it looks, and late-sown summer lettuces will survive light frosts with no problems. This means the plants will bear leaves all fall. This is not exactly "winter lettuce," but lettuce sown late in the growing season. (Gardner et al., 1972).

Winter lettuce tolerates low temperatures well, which allows it to be sown before winter (in October). (Hospido et al., 2009). The best lettuce is grown in the autumn at a nighttime temperature of 7 degrees Celsius. (Bonasia et al., 2023).

Lettuce provides the body with fiber and cellulose, improving digestion. It contains vitamins A, C, E, and K, as well as the minerals potassium, calcium, magnesium, iron, phosphorus, manganese, selenium, and zinc (Yang et al., 2022). Lettuce is also rich in thiamin, riboflavin, niacin, folate, pantothenic acid, and choline. This wealth of nutrients provides the body with numerous health benefits (Kim et al., 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 lettuce. 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 assessing lettuce accessions after harvesting in greenhouse cultivation. 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 differences of lettuce genotypes after harvest under uncontrolled conditions.

## **MATERIALS and METHODS**

### **Material**

Accessions of four standard lettuce varieties were investigated:

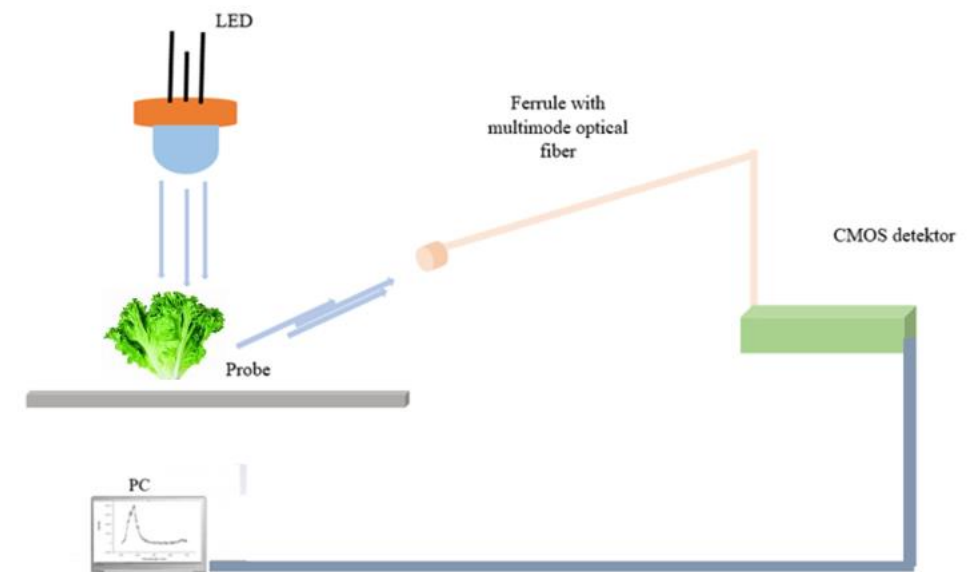
- **Paris White** - Medium early lettuce variety with upright, large heads, dark green leaves and creamy white core. With excellent tasty qualities, tender and aromatic. Recommended area for one plant – 25/30 cm
- **Lolo roca** - The variety has exceptional tasty qualities and is very suitable for fresh consumption. Tolerates adverse temperatures well. It can be grown in greenhouses or outdoors. Suitable variety for sowing by pre-prepared seedlings. You can sow every two weeks to maintain a constant crop of delicious lettuce. Vegetation period: 50-55 days.
- **Djentelina** is an early variety and can be grown year-round, in greenhouses or outdoors. It is characterized by a large, well-shaped rosette, with many curly light green leaves. The Gentilina salad is juicy, tender and very tasty and many useful substances. Its frequent consumption will bring many benefits to your body. The vegetation period is about 60 days. For spring production, the seeds can be sown at the end of January - the beginning of February in greenhouses. When the danger of frost has passed, they can be sown directly. For autumn production, seedlings are sown in September. For autumn production, seedlings are sown in September.
- **Hercules** - It is suitable for year-round cultivation with 55-65 days of vegetation. Its leaves are dark green and curly and form a large rosette. An extremely resistant variety to blight and downy mildew

The lettuce was grown using standard technology in a heated greenhouse in the winter. The seeds germinate at a temperature of 5–6 °C. The optimal air temperature is 20 °C. It is abundantly watered with lukewarm water two times a week. Lettuce was planted in a greenhouse in February. The depth of the planting holes should be 1.3 cm. The distance between the holes is 20 cm. 40 seeds per square meter are planted in previously abundantly moistened holes. Before germination, the soil was watered daily. Lettuce needs to be systematically fed with nitrogen fertilizers.

### **Fluorescence spectroscopy**

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

- A 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 2.4V and a current consumption of 0.02A. The minimum value of its reverse voltage is  $-6\text{ V}$ .
- 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  $4\text{ cm}^2$ . Its optical properties are to be transparent to visible light and to ultraviolet 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.



**Figure 1.** Mobile experimental installation used by fluorescence spectroscopy

- CMOS detector with sensitivity ranges from 200 nm to 1100 nm. Its 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.

#### **Study Indicators:**

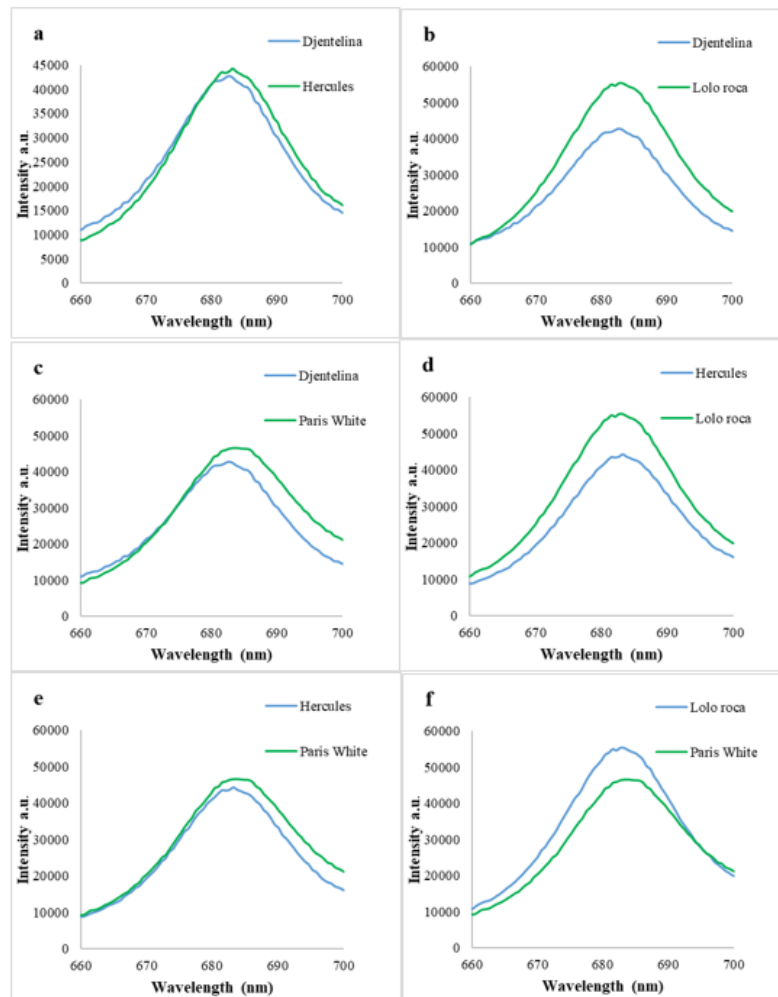
Spectral analysis - a spectral analysis of 15 leaves of each variety was carried out. The emission spectrum represents the wavelength distribution of an emission measured for a constant excitation wavelength. The excitation spectrum represents the dependence of the emission intensity measured for one 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

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



**Figure 2.** Difference in emission wavelength for Lettuce accessions of Paris White, Lolo roca, Djentelina and Hercules

The results conclude that fluorescence spectroscopy can be successfully applied as a rapid tool to establish the origin of unknown lettuce accessions in the presence of a rich spectra library. 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 compared to other varieties. The emission fluorescence signals of Paris White, Djentelina and Hercules (Figures 2 a, c, and e) are close in terms of wavelength localization and signal intensity level.

This is expected because the cultivars have a similar cell morphological composition when grown in a greenhouse. However, the method of fluorescence spectroscopy can be applied to distinguish the accessions of these three cultivars, since the correlation in the spectral distribution is sufficiently distinct and distinguishable to determine practically qualitatively the belonging of the accessions to a given cultivar. The method of fluorescence spectroscopy can practically be used to qualitatively determine the belonging of lettuce accessions to a given variety.

A literature review aimed at conducting such research It turned out that the experimental approach described so far for the method for determining varietal differences after harvesting in greenhouse cultivation of lettuce 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 in greenhouse-cultivated lettuces.

The method has been successfully applied to distinguish lettuce accessions from different varieties. Fluorescence spectroscopy can be applied to analyze carrot accessions of unknown cultivars and establish their origin with a sufficiently well-structured data library. Because it can be applied topically to trial samples. The application of the mobile circuit eliminates sample damage during transport and provides highly sensitive analysis.

## **Conclusion**

The fluorescence spectroscopy method is fast-acting in application as a field method in the determination of varietal differences after lettuce harvesting in greenhouse cultivation.

It has been proven that fluorescence spectroscopy will successfully apply as a rapid tool to establish the origin of unknown lettuce leaves in the presence of a rich spectra library. 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 the early, quick, and accurate differentiation of related lettuce 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 lettuce greenhouse cultivation.

### **Acknowledgement**

The accessions were produced and analyzed at Siika Naidenova's farm. I would like to thank all the farm staff for contributing to making this study possible.

### **REFERENCES**

- Bonasia, A.; Conversa, G.; Lazzizzera, C.; Elia, A. (2023) Harvest and Post-Harvest Performance of Autumn-Winter Butterhead Lettuce as Affected by Nitrogen and Azoxystrobin Application. *Agronomy* 2023, 13, 222
- Beattie W.R.(1940). Lettuce Growing, 61-107
- Gardner, B. R.; Pew, W. D. 1972. Response of Fall Grown Head Lettuce to Nitrogen Fertilization, 14-58
- Hospido, A., Canals, L.M., McLaren, S., Truninger, M., Edwards-Jones, G., Clift, R., (2009) The role of seasonality in lettuce consumption: a case study of environmental and social aspects *Int J Life Cycle Assess* 14:381–391
- Jung Kim M., Moon, Y., Janet C., Mou, B., Waterland, N. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.) *Journal of Food Composition and Analysis*, 49, 19-34
- Mou, B. (2008). Lettuce Handbook of Plant Breeding book series (HBPB, volume 1), 75–116
- Mou, B. (2009). Nutrient Content of Lettuce and its Improvement *Current Nutrition & Food Science* 5, 242-248
- Panwar, A. S., Prusty, A. K., Kashyap, P., Singh, M. P., Shamim, M., Verma, N., Jat, P. C., Kumar, S., and Chahal, V.P. (2021). Farming systems approach for ensuring food and nutrition security. *Indian Farming*. 71(10), 63–68
- Tudela, J., Hernández, N., Pérez-Vicente, A., Gil, M., I. (2017). Growing season climates affect quality of fresh-cut lettuce. *Postharvest Biology and Technology*, Volume 123, 60-68
- Worth, L.B., Rogers, M.A., Flavin-Hodge, C. and Schweser, G. (2023). Deep winter greenhouses for organic production of leafy greens in the northern United States. *Acta Hort.* 1377, 9-24
- Yang, X., Gil, M., Yang, Q., Barberian, F. (2022). Bioactive compounds in lettuce: Highlighting the benefits to human health and impacts of preharvest and postharvest practices *Comprehensive reviews in food science and food safety*