Use of VIS-NIR system combined with multispectral image analysis in Spinach (*Spinacia oleracea* L.) seed

M. Halkjær Olesen,[a] J.M. Carstensen[b] and B. Boelt[a]

[a]*Department of Genetics and Biotechnology, Aarhus University Forsøgsvej 1, DK-4200 Slagelse, Denmark. E-mail: merete.olesen@agrsci.dk*
[b]*Department of Mathematical Modelling, Technical University of Denmark, DK-2800 Lyngby, Denmark*

Introduction

Commercial seed samples are tested to ensure genetic and analytical purity, germination and vigour and seed health. To ensure uniform tests standardized protocols, workshops and proficiency tests are set up by The International Seed Testing Association.[1]

Seed health tests refer to the detection of disease-causing organisms such as fungi, virus or bacteria. A common seed health test in spinach is the freezer blotter test, where, after inoculation and incubation, the seeds are evaluated under microscope.[2] Performing such a test is very time consuming and the evaluation may vary between laboratories.

Near infrared spectroscopy has been used for fungi detection in different crops,[3,4] and in a pathogenic study with spectral image analysis, it was possible to differentiate between nine species of *Penicillium* species plated on agar.[5] These examples illustrate the possibilities of using spectroscopy and digital imaging for extracting precise information from the surface of pathogens. The separation includes colour distribution, colony dimension and texture measurement.

The objective of this study is to evaluate the potential for using multispectral image analysis in seed health tests of spinach with the focus on differentiating between infected and uninfected seeds, and between the five most common fungi on spinach.

Materials and methods

Prior to image analysis, seeds of spinach were sterilized and inoculated with one of five known fungal isolates, so that each seed was infected with only one fungal species. After inoculation, the seeds were placed in Petri dishes with wet filter paper, incubated for 24 h at room temperature, 24 h at minus 18°C and in a room germinator at 22°C with a 12 h/12 h day/night cycle for 7 days.

For image analysis the VideometerLab instrument (Videometer A/S, Denmark) was tested. It is equipped with a camera inside an integrating sphere lighted by led (light-emitting diodes) centred at the following 19 wavelengths 395, 430, 450, 470, 505, 565, 590, 630, 645, 660, 700, 850, 870, 890, 910, 920, 940, 950 and 970. The system was calibrated with respect to colour, geometry and self-illumination, thereby gaining a set of directly comparable images.

In each image there was a Petri dish with three uninfected seeds and three seeds infected with each of the five different fungi.

Data analysis was carried out using VideometerLab software and Matlab version 7.7.0. To ensure the same number of pixels were used for comparison in all analyses, areas of 18×18 pixels were chosen and referred to as the Region of Interest (ROI). Canonical discriminant analysis (CDA) was used for transformation of data, in order to minimize the distance to observations within the classes, and to maximize the distance to observations between classes. Ten images with three seeds from each group were used for supervising the model. Subsequently, images with inoculated seeds were examined after applying the supervised CDA transformation. Data were

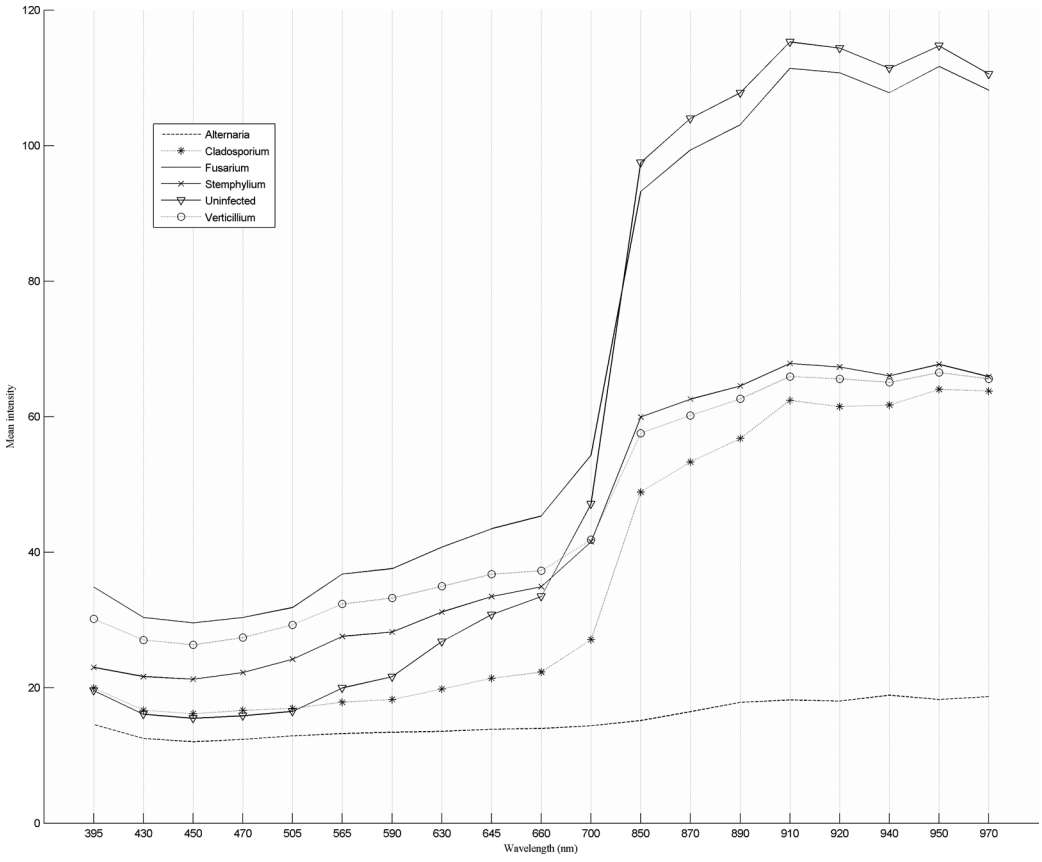

Figure 10.1. Mean value of multispectral images of one region of interest (ROI) of 18×18 pixels on each seed.

evaluated by plotting the intensity of the mean spectra for each of the six classes. Jeffries–Matusita distance was calculated after CDA transformation and used for pair-wise class separation.

Results

Mean intensity of the seeds at each of the 19 wavelengths (Figure 10.1) showed that all six classes were close to each other in wavelengths <700 nm. In NIR wavelengths the classes grouped into three with *Alternaria* having the lowest mean intensity, *Stemphylium*, *Cladosporium* and *Verticillium* intermediate and *Fusarium* and uninfected seeds with the highest pixel intensity.

The Jefferies–Matusita (JM) distance for VIS-NIR images (Table 10.1) shows that nine out of 15 pairs have a complete separation of the two distributions, as illustrated by the histograms in Figure 10.2. The data of uninfected seeds show complete separation from the fungi-infected seeds except from *Fusarium*, which has a minor overlap, shown by a lower JM distance. *Verticillium* and *Stemphylium* have a JM distance of 56.3%, which indicates that they are more difficult to separate.

JM distance based only on the wavelength in the NIR area illustrates almost the same, except for uninfected seeds separated from *Fusarium,* which has a much lower value, and *Stemphylium* separated from *Verticillium* with a higher value. Opposite results are found in the distribution based on wavelengths from the visible light, where the JM distance of *Fusarium* and uninfected seeds is 100%. Lower values are found by comparison of *Stemphylium*, *Verticillium*, *Fusarium* and *Cladosporium*, which indicates that it is more difficult to separate these when only VIS wavelengths are used in the measurements.

Table 10.1. Jefferies–Matusita distance converted to percent, calculated on CDA transformed data from VIS-NIR images (19 bands from 395 nm to 970 nm), data in () and underlined is from NIR images (8 bands from 850 to 970 nm) data with only () from VIS images (11 bands from 395 to 700 nm).

	Uninfected	*Alternaria*	*Cladosporium*	*Stemphylium*	*Verticillium*
Alternaria	100 (100) (100)				
Cladosporium	100 (100) (100)	100 (100) (100)			
Stemphylium	100 (100) (99,4)	100 (100) (100)	90,6 (96,6) (51,0)		
Verticilium	100 (100) (100)	100 (100) (100)	96,8 (97,2) (68,8)	56,3 (86,6) (30,8)	
Fusarium	99,6 (18,4) (100)	100 (100) (100)	100 (100) (100)	100 (99,8) (68,3)	93,1 (88,7) (82,1)

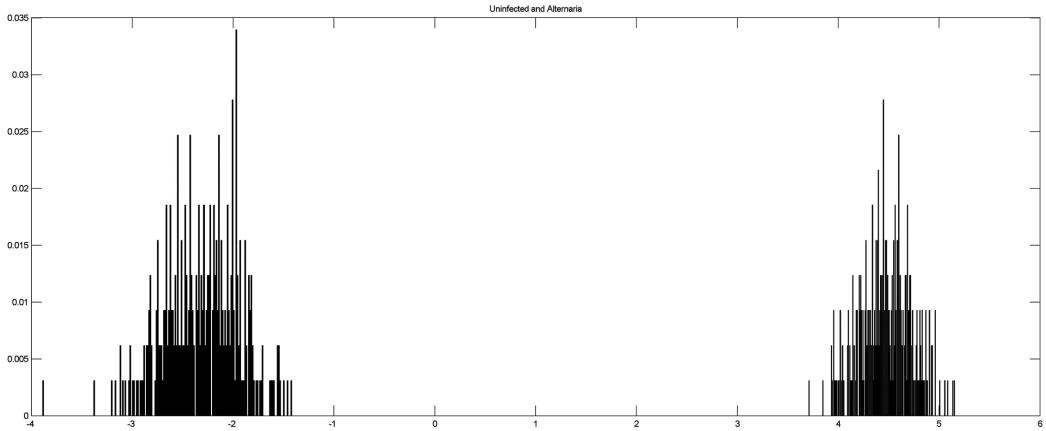

Figure 10.2. Histograms showing complete separation of features in a distribution of two CDA transformed VIS-NIR images.

Discussion

The study indicates that an algorithm to detect fungi on spinach seed can be developed and multispectral imaging is a promising tool to differentiate uninfected seed from infected seed. Separation between the five fungal species needs a combination of data from measurements of both VIS and NIR wavelengths. The most difficult fungi to separate with our methods are *Stemphylium* from *Verticillium*. In further studies it is expected that the textural difference in features between those two fungi can be used for separation.

The prevalence of the method used in this study illustrates the possibility of extracting precise information from the surface of heterogenic samples like seed and fungi colonies. The potential of combining spectral analysis and imaging has also been proven in a study of infected wheat kernels by hyperspectral imaging.[4] In this study they were able to separate fungal infected kernels from uninfected kernels. The need for analysis of the seeds at both the VIS and NIR wavelengths was also illustrated in a study with soybean seed. Here the classification accuracy was more than 99%, when they separated infected seeds from uninfected seeds, using spectroscopy in wavelengths from 490–1690 nm.[3] Further studies will highlight the possibilities for distinguishing between different stages within the development of fungal species, to see how early it is possible to detect the fungi. An additional step is to distinguish the fungal species when more than one species is present on each seed and compare the results with natural infected seeds.

To take the pictures with the Videometer, and to run the evaluation session takes less than 2 minutes. It is still a new technology that is not yet developed for practical use in spinach. Introduction of an instrumental method, as a replacement of visual microscopy, should improve the reproducibility of the seed health testing system between laboratories.

Acknowledgements

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