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Author for correspondence:
Birte Boelt, Email: Birte.Boelt@agro.au.dk

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Multispectral imaging – a new tool in seed quality assessment?

Birte Boelt[1], Santosh Shrestha[1], Zahra Salimi[1], Johannes Ravn Jørgensen[1], Mogens Nicolaisen[1] and Jens Michael Carstensen[2,3]

[1]Department of Agroecology, Aarhus University, DK-4200 Slagelse, Denmark; [2]Videometer A/S, DK-2970 Hørsholm, Denmark and [3]Technical University of Denmark, DK-2800 Lyngby, Denmark

Abstract

Multispectral imaging is a new technology that is being deployed to assess seed quality parameters. Examples of applications in the detection and identification of fungi on seeds are presented, together with an example of the technology used for maturity determination in sugar beet seed. Results from multispectral imaging are compared with reference methods, and a high correlation is found. Applications of the technique for varietal discrimination and insect damage are also presented. There is a need for non-destructive, reliable and fast techniques, and it is concluded that multispectral imaging has potential for seed quality assessment, in particular for those components associated with surface structure and chemical composition, seed colour, morphology and size.

Introduction

Seed quality is a multiple component characterization of seeds, including varietal and analytical purity, germination capacity, vigour, seed health and uniformity. Currently, testing for seed quality relies on physical and chemical as well as visual inspections, which are costly and time consuming. Furthermore, visual inspections are subjective and difficult to reproduce.

Visible features like colour, surface structure, morphology and seed size are determined by machine vision, primarily based on RGB (red, green and blue) cameras. Another optical sensing technology is near-infrared spectroscopy (NIRS) for the determination of chemical composition, both on the seed surface and internally. Hyperspectral imaging combines the two technologies and provides information on both spatial and spectral aspects (Huang *et al.*, 2015). Rahman and Cho (2016) have reviewed a number of non-destructive measurement techniques including machine vision, spectroscopy and hyperspectral imaging for the assessment of quality parameters of agricultural seeds. In particular, they found hyperspectral imaging to be a promising tool for seed quality aspects, as it combines machine vision and spectroscopy into one instrument. Applications of hyperspectral imaging in seed quality assessment are variety identification and classification, quality grading, detection of insect damage and fungi infection, and prediction of chemical composition (Huang *et al.*, 2015). The spectral regions employed cover a broad range of spectra in the visible (380–780 nm) and NIR (780–2500 nm) region (Huang *et al.*, 2015; Rahman and Cho, 2016). However, adoption of hyperspectral imaging by the seed industry requires a decrease of costs without compromising the accuracy of analysis (Dell'Aquila, 2007; Huang *et al.*, 2015; Rahman and Cho, 2016).

Selecting only those spectra providing specific information about the quality aspect in question is one way of reducing costs, yet still maintaining the high quality assessment. Multispectral imaging (MSI) systems include light sources providing a restricted number of wavelengths, which will provide the specific spectra. Examples are reported from the food industry where the spectra from four wavelength bands provide 100% correct classification of damaged mushrooms (Esquerre *et al.*, 2012), and similarly Xing *et al.* (2010) were able to distinguish sprouted from non-sprouted wheat kernels by the use of four specific wavelength bands.

The Organisation for Economic Co-operation and Development (OECD) defines the standards for the certification of seeds in the international trade, and the International Seed Testing Association (ISTA) develops, approves and disseminates methods for harmonized and standardized seed quality analysis. The Association of Official Seed Analysts (AOSA) has a similar function in the USA and Canada. The procedures of seed quality assessments are consolidated and globally accepted, and new technologies need to be thoroughly documented before approval is obtained. Both ISTA and AOSA recognize the need for new technologies for non-destructive, fast and reliable seed quality assessment to improve seed quality analysis and to reduce the cost of labour-intensive tests.

This review provides examples of applications of MSI for the assessment of physiological characteristics and determination of fungal infection. In addition, the results obtained by MSI in seed quality assessments are reviewed.

Seed development and maturity

Crops like sugar beet (*Beta vulgaris*) and spinach (*Spinacia oleracea*) have an indeterminate flowering pattern, which consequently leads to a non-uniform seed development and maturity pattern. Furthermore, the climatic conditions during seed production may affect the interval from anthesis to harvest time, resulting in seed lots with non-uniform maturity levels. Distinguishing empty or underdeveloped seeds from fully matured seeds is hardly possible by visual inspection, but their effect on the germination potential of a seed batch is significant (Tekrony and Hardin, 1969). In many studies on sugar beet seed performance, the underdeveloped seeds are considered to be the predominant reason for poor field establishment (TeKrony, 1969; TeKrony and Hardin, 1969; Snyder, 1971; Śliwińska *et al.*, 1999). Sugar beet is an important commercial biennial crop with a global production of 185 million metric tons in 2017 (USDA, 2017). It is direct-seeded and the seed quality greatly affects field emergence uniformity and crop yield (Agrawal and Rakwal, 2012).

During maturation, the amount of chlorophyll in the seed coat of many seed species decreases (Dell'Aquila, 2009), and Jalink *et al.* (1998) reported chlorophyll fluorescence (CF) as a non-destructive marker for seed maturity and performance in white cabbage (*Brassica oleracea*). Following this finding, CF has been demonstrated to correlate with seed germination in pepper (*Capsicum annuum*) and spinach (Deleuran *et al.*, 2013; Kenanoglu *et al.*, 2013).

X-ray provides a non-destructive evaluation method of the internal seed morphology associated with seed development (Dell'Aquila, 2009), and its application to predict germination capacity has been reported in bell pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*) seeds (van der Burg *et al.*, 1994; Gagliardi and Marcos-Filho, 2011; Gomes-Junior *et al.*, 2012; Silva *et al.*, 2013).

With the aim of comparing multispectral image analysis results from a non-uniform sugar beet seed lot, 20 sugar beet seeds in each of three maturity classes based on CF sorting according to Deleuran *et al.* (2013) were analysed by MSI technology. Multispectral images were captured by the VideometerLab multispectral imaging system (Videometer A/S, Hørsholm, Denmark). High resolution images (2056 × 2056 pixels) constituted images from 19 wavelengths ranging from 375 to 970 nm. The multispectral images of the sugar beet seeds were analysed by a normalized canonical discriminant analysis (nCDA) (Olesen *et al.*, 2015; Shrestha *et al.*, 2015) to discriminate between maturity classes. The mean reflectance from the surface of the sugar beet seeds in the maturity classes 1 to 3 were different across the 19 wavelengths. The immature seeds (class 1) generally had a higher reflection in the visible range of the spectrum, whereas all three classes showed differences in the NIR spectrum. Chlorophyll fluorescence is correlated with the reflectance in wavelengths 435, 645 and 660 nm of this MSI system. As indicated in Fig. 1, the immature seeds have a higher reflectance compared with maturity classes 2 and 3 in those wavelengths, but the reflectance of classes 2 and 3 is almost overlapping in the visible spectrum.

In order to extract information from the multispectral images, 17 variables (representing seed shape, colour and binary features) were evaluated. The RegionMSImean calculates a trimmed mean of MSI transformed pixel values within the blob (each single seed) and RegionMSIthresh returns a relative number of pixels exceeding the threshold of MSI-transformation in blob region based on the nCDA model (derived from all three classes) in their setting. The output of the image analysis was exported for the statistical data analysis. Canonical discriminant analysis (CDA) in SAS (SAS, 9.3) was used as statistical method. CDA is a dimension-reduction technique related to principal component analysis and canonical correlation. By the use of a classification variable and several interval variables, the CDA derives canonical variables (linear combinations of the interval variables) that summarize between-class variation.

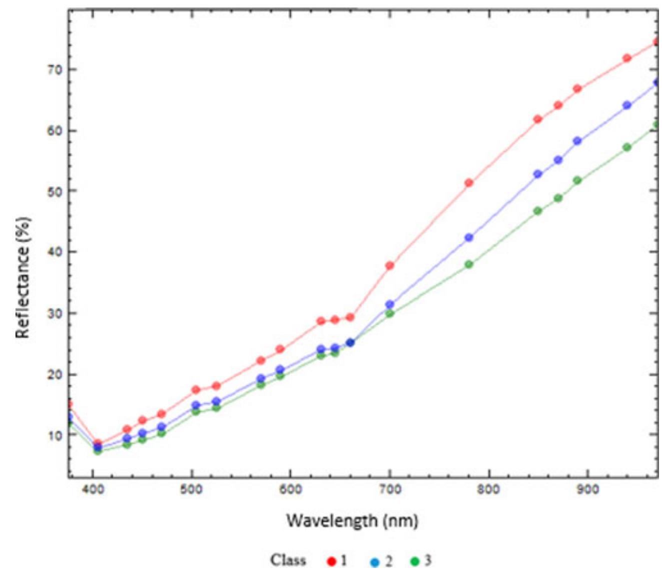

Figure 1. The mean reflectance (%) of the seed coat of sugar beet seeds in three maturity groups [group 1 (red): underdeveloped seeds; group 2 (blue): physiologically developed and ready for harvest; group 3 (green): seeds have passed physiological maturity].

For the characterization of differences between the three maturity classes, stacked photos of one seed per maturity class and a selected CT (computed tomography) slice from the 3D X-ray image of the same seeds were acquired (Fig. 2). North Star Imaging Europe, Inspection Services Group provided the CT scans. The seed development and maturity levels are illustrated in Fig. 2A with the least mature seed (class 1) to the left. In parallel with this, the slice of CT scan in Fig. 2B illustrates the internal structure of the seed, which is not yet fully developed and has free cavity space.

The CDA based on the surface characteristics from the MSI analysis classifies the sugar beet seeds into three distinctive classes (Fig. 3), which are identical with the three maturity levels from the CF reference analysis. The collection of seed colour, shape, texture and binary features identified three individual seed classes: (1) undeveloped, (2) physiologically developed seed, ready for harvest, and (3) seed past physiological maturity.

Figure 3 shows the scatter plot of the examined seeds classified into three classes according to the statistical analysis of the spectral information by plotting two derived variables of CDA (can1 and can2) for each assessed seed. Of the 60 seeds investigated in this study, 57 were classified in accordance with the CF classes, with a resulting 95% accuracy of classification in total. Comparing the MSI of each seed and the related CT scan (Fig. 2B) shows a good relationship between classification by the use of seed surface characteristics and seed development. MSI is therefore suggested as a reliable, non-destructive technology for the classification of seed development in sugar beet. The number of sugar beet seeds available, in particular the class 1, immature seed, did not allow for the characterization of germination capacity in this study. In castor bean (*Ricinus cimmunis*) MSI provided a model

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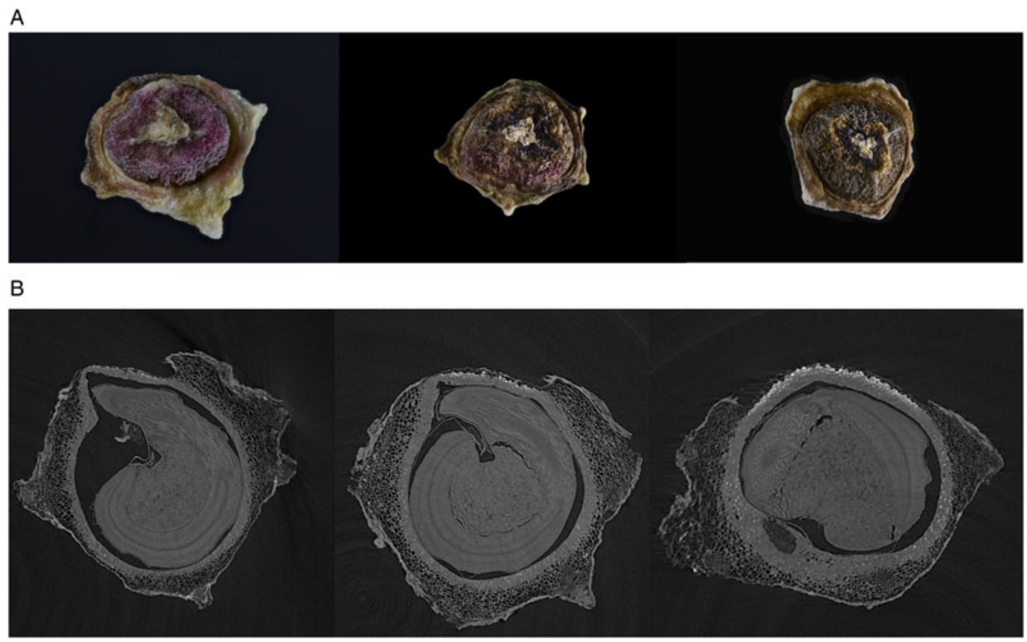

Figure 2. Three maturity groups of sugar beet seed. (A) RGB photo with maturity group 1 to the left; (B) slides of CT scan. Scans provided by North Star Imaging Europe, Inspection Services Group.

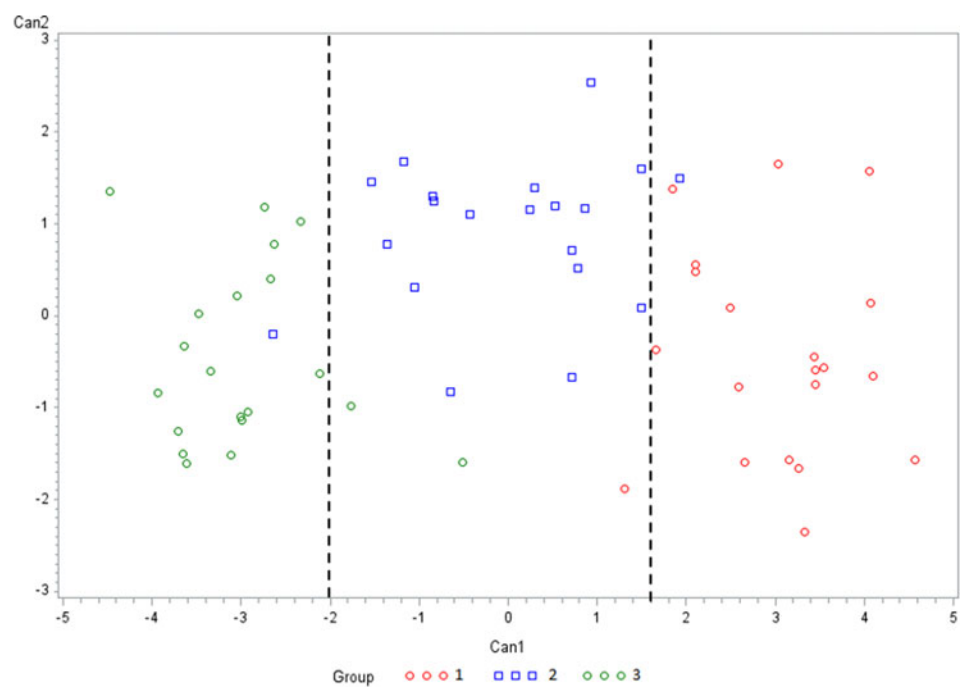

Figure 3. Classification of sugar beet seeds in three maturity groups (1–3) by plotting the can1 *versus* can2, derived from canonical discriminant analysis. Group 1: underdeveloped seeds, 2: physiologically developed and ready for harvest; 3: seeds have passed physiological maturity.

to predict viable from dead seed with an accuracy of 92% (Olesen *et al.*, 2015).

Multispectral imaging technology has been used in many studies for evaluating fruit maturity but not in seeds (Hahn, 2002; Lleó *et al.*, 2009; Baiano *et al.*, 2012; Rajkumar *et al.*, 2012; Qin *et al.*, 2013). However, evaluating seed maturity by different methods shows that differences in the level of embryo development as a probable effect of insects, other species pollen stimulation, application of synthetic hormones, extreme temperature or inappropriate nutrition condition of plant may affect seed quality (Gustafson, 1942; Hills, 1963; Tekrony and Hardin, 1969).

Seed health

Seed deterioration caused by fungi is an important factor in seed quality during storage, processing and propagation (Barton, 2016). Detection of fungi on seed is performed by inspection of the dry seed, washing tests, incubation methods, embryo count method or seedling symptom tests (Mathur and Kongsdal,

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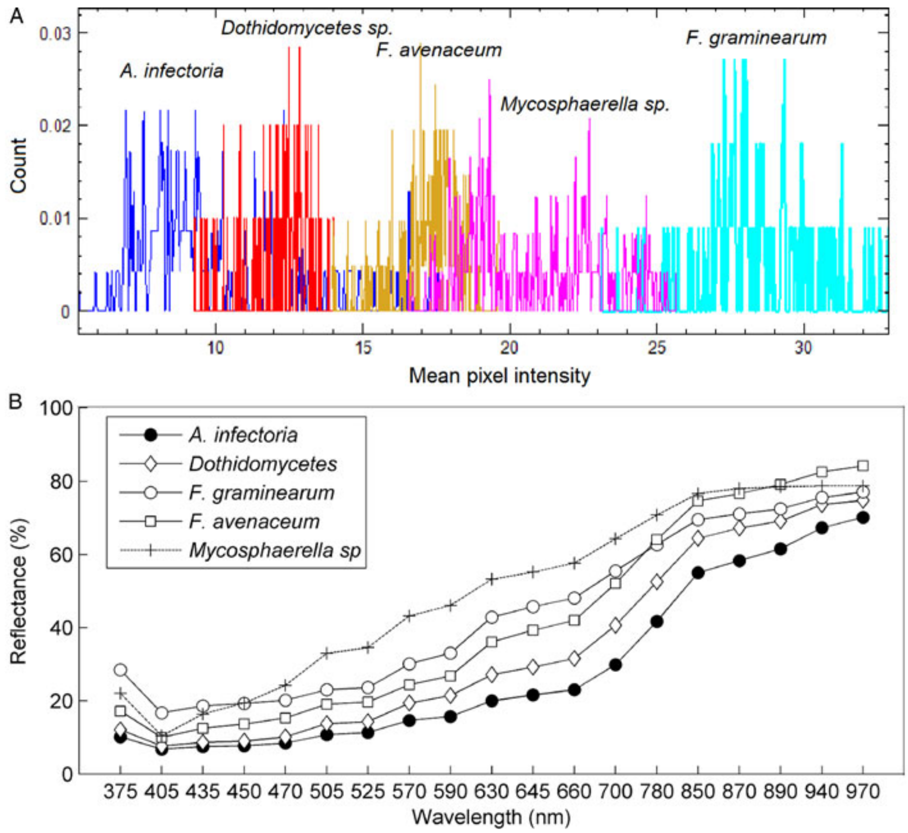

Figure 4. Mean pixel intensity (a) and mean reflectance (b) of barley seeds infected with *Alternaria infectoria*, *Dothidomycetes* sp., *Fusarium graminearum*, *Fusarium avenaceum* and *Mycosphaerella tassiana*.

2003). The preferred test method depends on the location of the spores, inoculum, etc. Furthermore, identification often requires sporulation and also expert knowledge (Lievens and Thomma, 2005).

The combinations of the features from multispectral images captured by visual light wavelengths and NIR wavelengths have proved to be useful in the separation of infected seeds from uninfected seeds in several species (Bodevin *et al.*, 2009; Olesen *et al.*, 2011; Jaillais *et al.*, 2015; Vrešak *et al.*, 2016). In spinach seeds, multispectral imaging has been used to discriminate uninfected seeds from seeds infected by *Stemphylium botryosum*, *Cladosporium* spp., *Fusarium* spp., *Verticillium* spp. or *Alternaria alternata* with 80–100% classification rate (Olesen *et al.*, 2011). The potential of using spectral characteristics of specific fungal species is to provide a fast optical screening method for fungal contamination of seeds; however, development of new methods depends on reliable reference methods.

Seeds of barley (*Hordeum vulgare*) can be infected by a high number of fungi, including pathogens such as *Fusarium graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum* and *Pyrenophora teres* but also a high number of non-pathogenic or slightly pathogenic taxa such as *Alternaria*, *Epicoccum*, *Cryptococcus* and *Dioszegia*. *Fusarium* spp. is a widely distributed fungus of high relevance in agriculture as it causes yield reductions in a range of agricultural crops, and many species in the genus produce mycotoxins responsible for serious quality deterioration. In malting barley, *Fusarium* also has a negative effect by causing gushing in beer (Christian *et al.*, 2011).

With the aim of comparing multispectral image analysis data with DNA-based data from infected barley seeds, approximately 200 barley seeds assumed to be infected by fungi from different origins (Europe) and years (2012–2016) of cultivation were tested by multispectral analysis and by next generation sequencing (NGS) of the ITS (Internal Transcribed Spacer) from total DNA (Nicolaisen *et al.*, 2014).

Prior to NGS evaluation, multispectral images of all the barley seeds were captured by a VideometerLab multispectral imaging system (Videometer A/S). A multispectral image consisted of images of 19 different wavelengths from visible to the near-infrared region in a range from 375 to 970 nm. Spectral information from the surface of seeds may be combined with information about size, shape and texture of the seeds for detection of fungal infection. The MS images of the barley seeds were analysed by an nCDA (Olesen *et al.*, 2015; Shrestha *et al.*, 2015) to discriminate fungi. A more detailed description of nCDA data analysis can be found in Shrestha *et al.* (2018) and Olesen *et al.* (2015).

From the NGS, approximately 2–4000 reads were obtained from each seed and these were subsequently identified to species level. The main fungal genera identified were *Fusarium*, *Pyrenophora*, *Epicoccum*, *Didymella*, *Alternaria*, *Bipolaris* and *Microdochium*. The fungal composition and quantities on each seed varied significantly. Some seeds were infected mainly by a single fungus and some were infected by multiple fungi. A total of 48 seeds primarily infected by a single fungus species viz. *Alternaria infectoria*, *Dothidomycetes* sp., *Fusarium graminearum*, *Fusarium avenaeum* and *Mycosphaerella tassiana* were used for the multivariate data analysis to identify the causal fungal species.

The barley seeds infected with the different fungal species showed considerable variation in mean pixel intensities (Fig. 4a). The seeds infected with *F. graminearum* and *Mycosphaerella tassiana* had distinct differences in mean pixel intensities (Fig. 4a). The remaining infected seeds also showed variations, although the mean intensities of groups overlapped each other. A similar trend observed in Fig. 4b was that the

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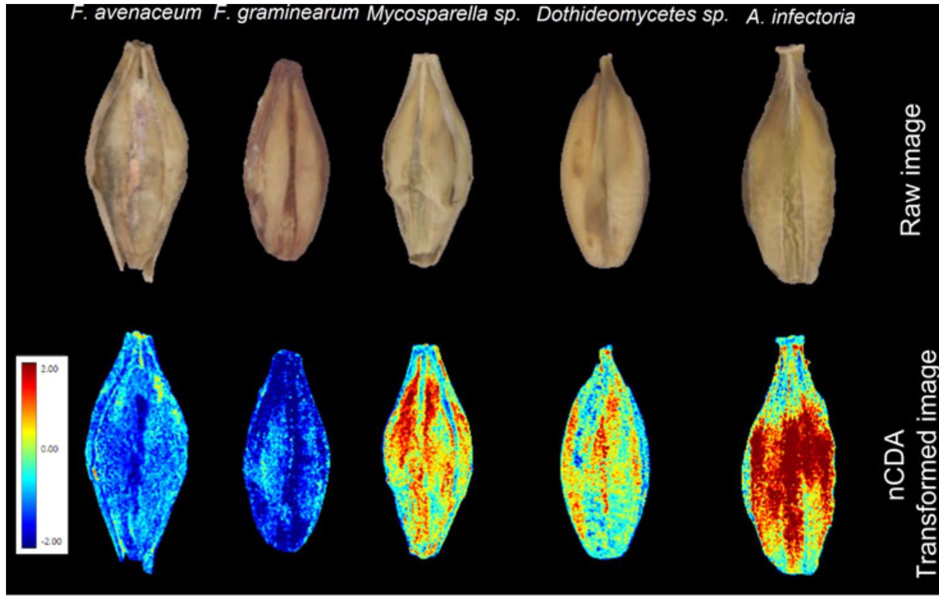

Figure 5. Raw images of the barley seeds and corresponding transformed image by the nCDA model.

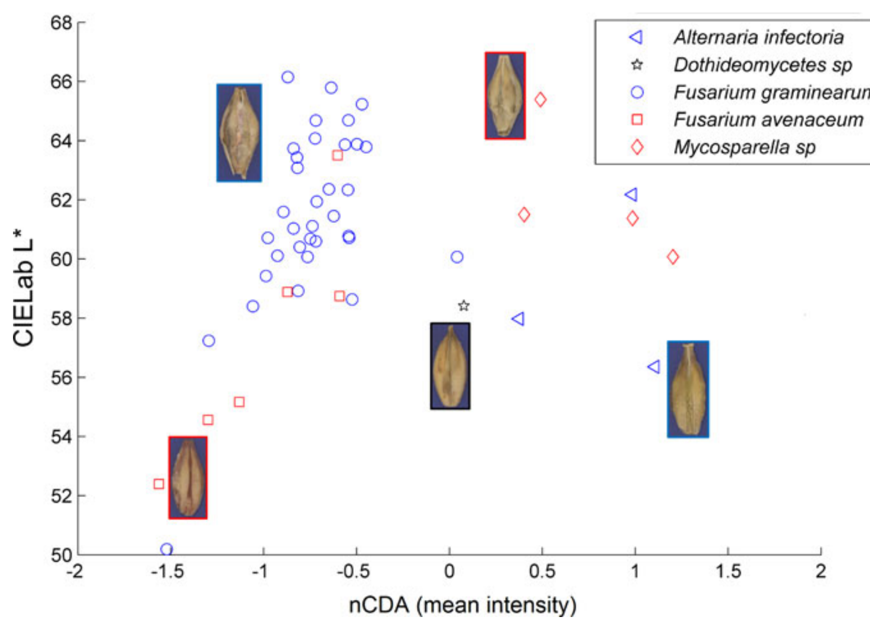

Figure 6. nCDA scores plot showing the clustering of the barley seeds infected by the five different fungi species. Each score is a mean intensity of the nCDA transformed seed image.

spectral signatures of the infected seeds had variation in mean reflectance. The variation in spectral signatures showed the possibility of developing a chemometric method for separating the infected seeds.

An nCDA analysis involving five classes (i.e. each fungus class) of infected seeds showed differences among the infected seeds (Fig. 5). Each pixel of an nCDA-transformed image represented a score calculated by the nCDA model. It determined the score in a way that maximized variation between the classes and reduced variation within a class (Olesen *et al.*, 2015). The nCDA model showed contrasting differences between *Fusarium* spp. and others (Fig. 5). The nCDA model did not show significant differences between the two *Fusarium* spp.; however, a colour feature CIELab L*, which relates to the brightness/darkness, showed a tendency of separation between them (Fig. 6). The results suggest the possibility of using multispectral imaging for identifying different fungal species in barley seeds. However, the nCDA model can be significantly enhanced by using additional steps such as hierachical/step-wise factorial classification or pairwise comparisons (Shrestha *et al.*, 2015; Kimuli *et al.*, 2018) to acquire distinctive discrimination among the fungal species. For instance, a step-wise classification between the two *Fusarium* species can significantly increase the separation, as shown in Fig. 7.

A proper validation of a developed model is essential for a multivariate image analysis to assess the robustness and reproducibility of the results (Shetty *et al.*, 2011). However, the nCDA model could not be validated due to availability of only few and disproportionate samples of the five fungal infected seeds. Previous studies have shown the potential of multispectral imaging in detecting or identifying a specific fungus species (Bauriegel *et al.*, 2011; Dammer *et al.*, 2011; Jaillais *et al.*, 2015; Leplat *et al.*, 2018) in wheat seeds with high accuracy. The study dealt with identifying several fungal species in barley seeds. The seeds examined had a diverse background such as

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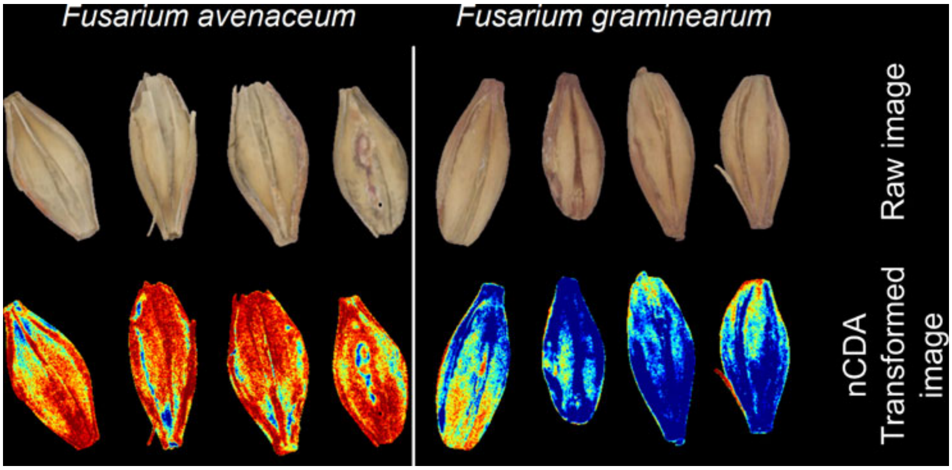

Figure 7. A stepwise nCDA classification of *Fusarium avenaceum* and *Fusarium graminearum* (top: raw image; bottom: subsequent transformed image).

genetic (different varieties), environment (year and region of cultivation) and ageing apart from the fungal infection. These factors have an influence on the discriminatory ability of the imaging system (Shrestha *et al.*, 2016). Considering this, the results obtained are promising and show the prospect of developing a method that can identify several fungal infections in seeds simultaneously.

Other applications of multispectral imaging

Varietal purity is a very important aspect in plant breeding programmes, seed production, genebank management and in the trading of seed in general. Therefore, any non-destructive, fast and reliable method for the identification and discrimination of varietal purity will be of interest. Recent studies have shown the potential of MSI combined with chemometric methods for the varietal identification of transgenic *Bacillus thuringiensis* rice seeds (*Oryza sativa*) and the discrimination of two varieties, respectively conventional and glyphosate-resistant soybean seeds (*Glycine max*) and their hybrid descendants (Liu *et al.*, 2014, 2016). In tomato, Shrestha *et al.* (2015) examined eleven varieties/accessions either all together or pairwise with their offspring for varietal identification and discrimination. By the use of MSI and multivariate data analysis the classification accuracy obtained was between 100 and 86%. International genebanks handle a large number of seed accessions and the evaluation of seed phenotype is usually done by visual inspection. Seed accessions are evaluated on arrival to the genebank to determine if the accession is new or already exists in store. Furthermore, the phenotype is evaluated when accessions have been regenerated to assure that the newly harvested seed is true to type to the stored sample. These procedures are very time consuming and require highly skilled and patient staff. Hansen *et al.* (2015) demonstrated that MSI could classify 20 diverse rice varieties with 93% accuracy.

A further application of MSI is the determination of insect damage (Ma *et al.*, 2015; Sendin *et al.*, 2018) and other crop and weed seeds (Sendin *et al.*, 2018) have recently been documented.

Concluding remarks

From the applications presented and reviewed, it is concluded that MSI has a potential for seed quality assessment, in particular for those components associated with surface structure and chemical composition, seed colour, morphology and size. MSI is a non-destructive, reliable and fast tool with a potential for the detection and characterization of fungi, insect damage, varietal purity and components associated with seed development and quality aspects.

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