Vis-NIR imaging – A tool to predict germination of spinach seed

Merete Halkjaer Olesen*, Nisha Shetty, Lise Christina Deleuran, Rene Gislum and Birte Boelt

Aarhus University, Faculty of Agricultural Sciences, Department of Genetics and Biotechnology, Forsøgsvej 1, DK-4200 Slagelse, Denmark
*Corresponding author: merete.olesen@agrsci.dk

Introduction

Spinach (*Spinacia oleracea* L.) seeds germinate relatively slowly and therefore fast non-destructive methods to predict germination are required. Earlier studies of spinach seed germination at different temperatures indicated that variation in germination can be caused by physical and chemical properties in the pericarp.[1-3] Studies of *Brassica* species illustrate that it is possible to predict the germination quality of a seed lot by determination of the chlorophyll level in the seed coat.[4] A new approach is to use vision systems to identify the surface properties of seeds.[5] Multispectral imaging has been used in the assessment of seed quality, e.g. fungal infection in wheat[6] and spinach.[7] Near infrared (NIR) spectroscopy has been used to test seed vitality and vigour[8-10] and visual imaging has been used to follow the development of seeds during germination.[5] Use of imaging as a non-destructive analysis enables analyses to be performed in the process lines and could be of great interest to the seed industry. Thus, the purpose of this preliminary study was to examine how surface properties of spinach seeds, as determined by multispectral imaging, can be correlated to the ability to germinate.

Materials and Methods

Samples

Seeds from a commercial spinach cultivar were harvested 3, 2, 1 and 0 weeks before crop maturity in July 2009 and divided into different seed fractions depending on chlorophyll level and seed size (<2.5 mm, 2.5-3.25 mm, 3.25-3.75 mm and 3.75-4.25 mm). This preliminary study was based on seeds harvested 2 weeks before crop maturity and with seed size below 2.5 mm.

Imaging and germination

From each seed fraction, four replicates of 25 seeds were used to capture digital images by the VideometerLab instrument (Videometer A/S, Hørsholm, Denmark). The VideometerLab instrument is equipped with a camera inside an integrating sphere illuminated by 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 nm. The integrating sphere ensures that light is scattered evenly around the object, giving uniform diffuse lighting. The diodes emit a narrow band of light that ensures that only light of the desired wavelength is present when an image is acquired. The system is calibrated with respect to colour, geometry and self-illumination, thereby gaining a set of directly comparable images.

Images of spinach seeds were acquired right after the seeds were sown in Petri dishes lined with wet filter paper. Afterwards the Petri dishes were placed in a germination cabinet at 15°C and a 8/16 day/night cycle was followed (ISTA, 1996). After 3, 7, 14 and 21 days seeds were visually inspected for germination. The following characters for germination were assigned: 0 - no visible germ, 1 - germ <0.3 mm; 2 - germ 0.3-1.0 mm and 3 - germ >1.0 mm.

Data analysis

One captured image with 25 seeds (10 non-germinated seeds and 15 germinated seeds) was chosen for the preliminary study presented in this paper.

Texture and greyscale data were extracted from the image using a macro written in the free software ImageJ 1.43 at http://rsbweb.nih.gov/ij/. The multispectral image was imported as a raw image and the output data from the macro was a table that contained grey level and texture data for each seed. Data from the table was further analysed using MATLAB (version 7.7.0. R2009b; The MathWorks, Inc., Natick, MA, USA) with the ECVA_Toolbox (version 2.01; available at http//www.models.life.ku.dk).

Principal component analysis (PCA) was performed to explore the data for extreme samples and to observe groupings according to non-germinated and germinated seeds. The supervised classification method called extended canonical variates analysis (ECVA)[11] was tested. ECVA was performed using all collected

Reference paper as:
Olesen, M.H., Shetty, N., Deleuran, L.C., Gislum, R. and Boelt, B. (2012). Vis-NIR imaging – A tool to predict germination of spinach seed, in: Proceedings of the 15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 74-77.

the wavelengths (Vis+NIR) as well as separately on visible and NIR wavelength regions. Prior to analysis, the data was scaled block-wise (mean data was standardised according to each block, here blocks are features of the image). The model was validated using random cross-validation with 10 iterations and 5 segments.

Results and Discussion

Classification of germinated and non-germinated seeds by imaging could be done using grey level mean and texture features from images acquired by diodes centred at selected NIR wavelengths.

Exploratory analysis of data by a PCA score plot did not result in grouping of the seeds. The best results were obtained when a proper classification method, ECVA, was applied to only NIR data (Figure 1). Both visible and NIR data in one analysis or only visible data resulted in a higher number of misclassified seeds (Figure 1).

ECVA was tested on a different dataset obtained by feature extraction, which results in the best separation using mean and inverse difference moment data (Figure 1 and 2). An ECVA model based on the NIR data alone correctly classified 24 of 25 seeds germinated at 15°C for 21 days. The present results are in line with earlier studies in which data from single seed NIR measurements were used for classification of spinach (*Spinacia oleracea* L.),[10] wheat (*Triticum aestivum* L.),[12] pine(*Pinus patula* Shiede and Deppe)[8] and beechnuts (*Fagus orientalis* Lipsky).[13]

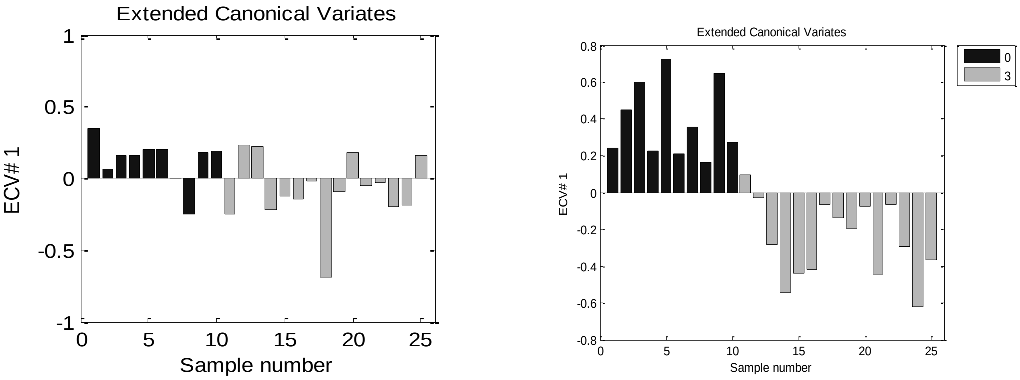

Figure 1. Bar plot of mean data: visible wavelengths left and NIR wavelengths right. Extended canonical variance component 1 (ECV#1) against sample number. Bars with positive value were classified as non-germinating seeds and bars with negative value were classified as germinating seeds. Black bars represent non-germinated, and grey bars germinated seeds in the final germination test.

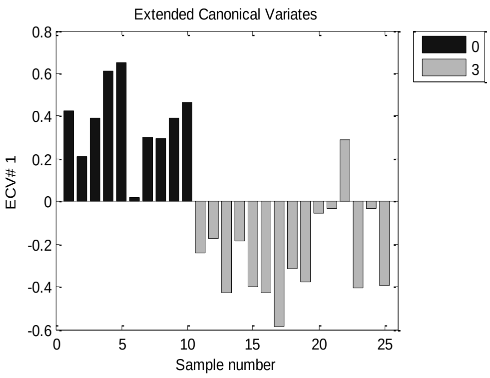

Figure 2. Bar plot of inverse difference moment data. Extended canonical variance component 1 (ECV#1) against sample number. Bars with positive value were classified as non-germinating seeds and bars with negative values were classified as germinating seeds. Black bars represent non-germinated, and grey bars germinated seeds in the final germination test.

Conclusion

Multispectral imaging is a potential tool for prediction of germination ability in sorting small seeded fractions of spinach. Images were captured with both visible and NIR wavelengths. After feature extraction and ECVA data analysis the best results were obtained from images captured by near infrared wavelengths using mean and inverse difference moment data.

Reference paper as:
Olesen, M.H., Shetty, N., Deleuran, L.C., Gislum, R. and Boelt, B. (2012). Vis-NIR imaging – A tool to predict germination of spinach seed, in: Proceedings of the 15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 74-77.

Acknowledgements
Thanks to the Danish Seed Council, the Ministry of Food, Agriculture and Fisheries, and the Danish Food Industry Agency for providing financial support.

References
1. J.G. Atherton and A.M. Farooque, **19**, 25-32 (1983).
2. N. Suganuma and H. Ohno, *J. Jap. Soc. Hortic. Sci.* **53**, 38-44 (1984).
3. D.I. Leskovar, V. Esensee and H. Belefant-Miller, *J. Am. Soc. Hortic. Sci.* **124**, 301-306 (1999).
4. H. Jalink, R. v. d. Schoor, Y.E. Birnbaum and R.J. Bino, *Patent* (2000).
5. A. Dell'Aquila, *Seed Sci. Technol.* **35**, 519-538 (2007).
6. C.B. Singh, D.S. Jayas, J. Paliwal and N.D.G. White, *Trans. Asabe* **50**, 2171-2176 (2007).
7. M.H. Olesen, J. M. Carstensen and B. Boelt, *Seed Sci. Technol.* **39**, 140-150 (2011).
8. M. Tigabu and P. C. Oden, *Seed Sci. Technol* **32**, 593-606 (2004).
9. M. Tai Gi and K. Woo Sik, *J. Kor. Soc. Hort. Sci.* **44**, 545-548 (2003).
10. M.H. Olesen, N. Shetty, R. Gislum and B. Boelt, *J. Near Infrared Spectrosc.* **19**(3), 171-180.
11. L. Norgaard, R. Bro, F. Westad and S.B. Engelsen, *J. Chemom.* **20**, 425-435 (2006).
12. R. Juhasz, S. Gergely, T. Gelencser and A. Salgo, *Cereal Chem.* **82**, 488-493 (2005).
13. A. Soltani, T.A. Lestander, M. Tigabu and P.C. Oden, *J. Near Infrared Spectrosc.* **11**, 357-364 (2003).

Reference paper as:
Olesen, M.H., Shetty, N., Deleuran, L.C., Gislum, R. and Boelt, B. (2012). Vis-NIR imaging – A tool to predict germination of spinach seed, in: Proceedings of the 15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 74-77.