Detection of physiological processes in wheat using near infrared spectroscopy

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Introduction

Sequences of biochemical, enzymatic and morphological changes occur during the so-called generative development in seed. To investigate the physiological status of seed, complicated chemical, biochemical, enzymatic, botanical and morphological test methods are needed and the results obtained are often inaccurate and obscure due to extensive biological variations. In order to examine the complex physiological events taking place in plant materials and to understand the biochemical basis of processes, near infrared (NIR) techniques can be applied. NIR is widely used in the quality control of cereals but also can be used as a tool in basic research, breeding programmes, technological process analysis and control and in detection of functional properties of cereal.[1]

According to Batten and Blakeney,[2] Downey[3] and Salgó *et al.*,[4] different physiological processes (ageing, maturation, germination) were followed in cereals using NIR. However, the application of NIR methods were sometimes critical because of the lack of proper reference methods and the fast dynamic changes in the investigated materials.

The aim of the present study was to observe the biochemical physiological changes in intact wheat seeds during seed development (maturation) using near infrared methods. The investigations were focused on the qualitative changes of spectral characteristics and of the main constituents in plant tissues.

Materials and methods

Six different wheat varieties with different harvest time were grown as field trials at the Agricultural Research Institute of the Hungarian Academy of Sciences (ARIHAS), Martonvásár.

The investigated varieties were as follows: GK Öthalom (early), Bánkúti 1201 (early), Jubilejnaja 50 (semi medium), Mv 23 (medium), Fatima (medium), Mv 15 (semi late).

Whole primary ear samples were collected twice or three times weekly from crops of each variety. The sampling began 12 days after flowering (DAF). Because of the high biological variabilities of seed materials six independent ear samples were collected each time from each variety.

The 16 sampling time (1–16 points) covered the whole 41 days long maturation period.

Wheat seed were prepared from ears manually directly after sampling and were scanned in intact form; their moisture content was measured immediately. The remaining fresh samples and the dried materials were frozen (–15°C). From each ear about 40–60 seeds were prepared and the average fresh weight of the samples (mg seed^{-1}) was measured.

Moisture content of wheat samples was determined in triplicates using 105°C and 4 h oven drying method.

Five independent spectroscopic scans were recorded from each sample. A static sample cup was used with a special ring for volume reduction. Scans were collected with an NIRSystems 6500 instru-
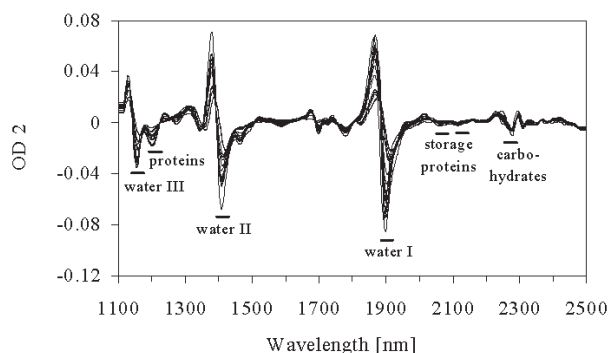
Figure 1. Second derivative spectra of wheat samples taken during maturation.

ment (NIRSystems Inc., Silver Spring, MD, USA) using sample transport module (reflection mode) in the 1100–2500 nm wavelength range, and the raw spectra were transformed, second derivatives (OD 2) were calculated.

Spectral and reference data were processed with the NSAS 3.30 (NIRSystems Inc., Silver Spring, MD, USA) software package.

Results and discussion

A very high level of moisture content was observed in the early lag period of maturation then a proportional but slow decrease was detected (data not shown). An extremely accelerated loss in moisture was measured after 38 DAF. This is the period of maturation where most of the synthesised materials were "dried" into the matrix and the "character" of water was changed significantly.

The changes which took place in wheat seeds were observed in the near infrared spectra. The reflectance spectra of maturing seed samples showed a very high variation in the whole wavelength range. This variation still remained after transformation (calculation of the second derivatives) of raw spectra (Figure 1). Characteristic regions were observed in the spectra where the changes of main constituents can be followed and analysed in details.

The water peak around 1900 nm (water I) was changed dramatically during maturation in all the six varieties. In the early stage of maturation (until 19 DAF) the water peak showed a 15 nm shift to the
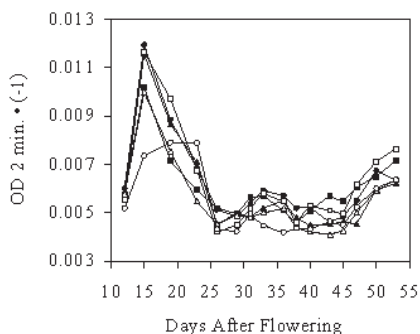

Figure 2. Change of local minimum values of carbohydrate peak (2270–2280 nm) during maturation.
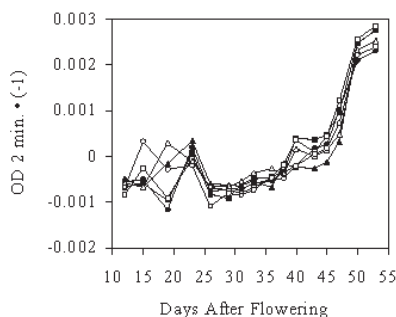
Figure 3. Change of local minimum values of protein peak (2050–2070 nm) during maturation.

lower wavelength value and about a 25% increase in absorbance (decrease in OD 2) showing the higher concentration of "free" type of water in seed. The level of "free" water decreased rapidly in the 23–25 DAF period and at the end of maturation (drying) a characteristic shift (15–20 nm) to a higher wavelength value in the water peak was observed. Similar changes were detected in the region of other water peaks around 1410 nm (water II) and 1155 nm (water III) respectively (data not shown).

The second derivative spectra confirmed a non-linear accumulation of oligo- and polysaccharide (mainly starch) components during maturation, which was concluded from the changes of a characteristic peak in the 2270–2280 nm region. (Local minimum values of the 2nd derivative spectra at 2260–2290 nm were drawn *vs* maturation time.) In the early phase of maturation (until 26 DAF) a huge amount of saccharides were synthesised and converted very fast (Figure 2) and the synthesis of the reserve polysaccharides was started at an accelerated speed. The accumulation of starch was extremely fast in the last period of maturation (45–52 DAF).

Bands of spectral region (2050–2130 nm), which were mainly associated with storage proteins (gliadins and glutenins), have been changed to high extent during maturation. An uncertain "shift" of protein bands (2050–2070 and 2100–2130 nm) until 23 DAF confirmed the formation of different elements (amino acids, peptides) for protein synthesis.[5] In the second phase of maturation (after 26 DAF) a linear and in two phase accelerated increase in the amount of storage proteins were observed (Figure
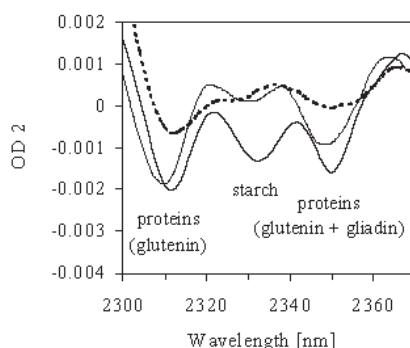

Figure 4. Change of characteristic protein and starch peaks at three different stages of maturation (dashed line = 19 DAF, thin line = 36 DAF, thick line = 53 DAF).

3). Similar results were obtained when the changes of another storage protein band (1190–1210 nm) was analysed.

To see the kinetic of the formation of seed reserves it is essential to follow the changes of peaks of proteins and starch *vs* maturation time (Figure 4). In the early phase of maturation only a limited amount of protein was present. After five days, an accelerated protein synthesis was observed while starch was produced only to a smaller extent. In the final period of maturation the synthesis of starch was very fast.

In order to be able to check the physiological changes or physiological status of seeds quantitatively, calibrations were developed for detecting moisture content, dry weight, nitrogen content and maturation time. Figure 5 summarises the statistical results of calibrations and tests. Moisture content was predicted with 2.42% standard error of prediction (*SEP*) value, which means a 5% coefficient of variation in a broad moisture range. The high correlation coefficient shows that maturation processes (changes in NIR spectra) are highly associated with water content. Calibration and tests carried out for detection of maturation time confirmed that there is information in the NIR spectra which is sensitive enough for predicting the physiological status of wheat seed with *SEP* = 2.4 days value. The coeffi-
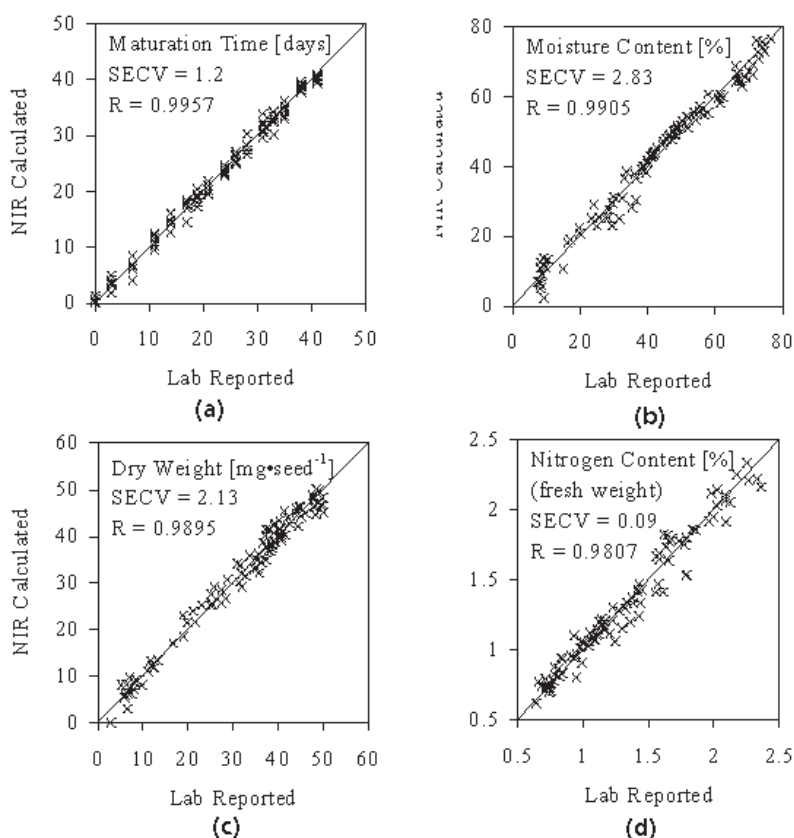

Figure 5. Model equations for determination of (a) maturation time, (b) moisture content, (c) dry weight and (d) nitrogen content.

cient of variation of this measurement was 5–7%, which is very acceptable considering the high biological variability of materials tested.

Conclusion

The seed maturation as a complex metabolic process can be sensitively followed by NIR spectroscopy. The details of NIR spectra are informative concerning the changes of macro components or their fractions and they have plenty of hidden chemical and biochemical information. The kinetic changes of the main constituents can be recognised in the spectra and based on spectroscopic results the physiological status of seed can be predicted quantitatively.

Acknowledgments

References

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