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Note

Fourier transform infrared – attenuated total reflection for wheat grain**

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A b s t r a c t. Mid-infrared regions of the spectrum of grain of four *Triticum* species were analyzed using Fourier transform infrared – attenuated total reflection. Significant variations were noted in the absorbance of all studied taxa over four wavenumber ranges. The principal component analysis supported strong discrimination of the four examined species. The percentage of variation explained by the first two principal component analyses reached 95.04%, including principal components 1-72.16% and 2-22.88%. The applied method supports quick identification of the grains of various hulled species of wheat and it is a useful tool for evaluating the seeds and food products obtained from those cereal species.

K e y w o r d s: wheat, grain, Fourier transform infrared – attenuated total reflection, mid-infrared spectroscopy

INTRODUCTION

A growing interest in foods delivering high nutritional value and health benefits prompts breeders to develop new grain species that meet consumer expectations. The grain of hulled wheats (spelt – *Triticum spelta,* emmer – *T. dicoccum* and einkorn – *T. monococcum*) and the resulting products meet the requirements set for functional foods (Stalknecht *et al.,* 1996; Suchowilska *et al.,* 2009). The species-specific phytochemical composition of the studied wheats requires the development of a quick and easy identification method. The proposed technique would contribute to genetic and breeding efforts, seed trade, determination of species identity and seed homogeneity for the needs of the food processing industry.

Contemporary breeding efforts rely on various instrumental techniques that support quick identification of breeding material and evaluations of their suitability for further stages of production. Selected methods produce very large quantities of data which are processed with the involvement of multidimensional techniques to precisely identify varieties and breeding lines. The above goals are attained through the classification and discrimination of the studied objects. Infrared spectroscopy is a reliable method for determining the concentrations of functional substances in plant and animal material (Schulz and Baranska, 2007). The most popular method of choice is NIR which supports quick and non-invasive determination of components such as proteins, fats, carbohydrates and water (Wehling, 2003). Mid-infrared spectroscopy (MIR) is a reliable and a relatively well researched method which is used in qualitative studies and quantitative determinations of various substances, including food products of plant and animal origin (Elmore *et al.,* 2007). One of the greatest advantages of MIR is the option of producing spectra for highly varied solids, fluids and gases. MIR spectroscopy supports a host of analytical applications, including analyses of fatty acid structure in plant and animal fats (Safar *et al*., 1994), evaluations of ethanol and sugar content of alcoholic beverages (Nagarajan *et al.,* 2006) and determinations of microelement concentrations in milk (Soyeurt *et al*., 2009). In conventional infrared spectrometers, a beam of radiation is transmitted directly through the analyzed sample. Fourier transform infrared – attenuated total reflection (FT-IR/ATR) is used when transmission spectroscopy fails to produce repeatable and reliable results

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(for example, in analyses of fluids) and when a given chemical compound has to be precisely identified. FT-IR/ATR spectra have clear and easily identifiable peaks that correspond to specific bonds and groups of bonds and functional groups (http://www.siliconfareast. com/FTIR.htm).The result is determined by the thickness of the sample that should not exceed several dozen micrometers. In the attenuated total reflectance (ATR) technique, the infrared beam is passed through transparent material with a high angle of refraction, mostly diamond. The above method supports very accurate measurements of infrared radiation intensity.

The objective of this study was to evaluate the effectiveness of the FT-IR/ATR technique in discriminating four *Triticum* species based on absorbance values for wavenumbers corresponding to the mid-infrared spectrum.

MATERIALS AND METHODS

The experimental material comprised the grain of 46 genotypes (Table 1) of *T. monococcum* (11 lines), *T. spelta* (10 lines), *T. dicoccum* (13 lines) and *T. aestivum* (12 cultivars) cultivated in an exact field experiment. The samples were ground using a laboratory mill (Cullati MFC, Zürich, Switzerland, with a mesh size of 1 mm) and were analyzed in the Vector 22 FT-IR (Bruker, Karlsruhe, Germany) spectrometer equipped with an MIR source. A horizontal ATR module (with an embedded diamond that guarantees three internal reflections, mounted directly on the ZnSe crystal) (SensIR-Technologies, USA) was mounted inside the spectrometer chamber. The ground sample was directly compressed against the surface of the diamond using a rotary pressure applicator with a slip clutch for reproducible pressure application. The applied detector was deuterated L-alanine doped triglycine sulfate (DLATGS). Each sample was scanned 32 times within the applied wavenumber range (650-4000 cm^{-1}) with the resolution of 2 cm^{-1}. The results were statistically processed. A paired t-test was performed to estimate the significance of differences in absorbance (ABS) between the studied species (*T. monococcum – T. dicoccum, T. monococcum – T. spelta, T. monococcum – T. aestivum, T. dicoccum – T. spelta, T. dicoccum - T. aestivum* and *T. spelta – T. aestivum)*. Wavenumbers producing significant differences in absorbance for all compared pairs were subjected to principal component analysis (PCA). Statistical analyses were performed with the use of STATISTICA software (StatSoft, Inc., 2008).

T a b l e 1. Genotypes of *Triticum sp.* examined in the experiment

Genotype	Source
T. monococcum	
PL 020790, PL 024068, PL 020751	NCPGR[1]
PI 290511, PI 326317, PI 330551, PI 352479, PI 418587, PI 584654, PI 428171	NGRL[2]
Terzino	CBRD[3]
T. dicoccum	
Cltr 9258, PI 164582, PI 244341	NGRL[2]
PL 020758, PL 021606, PL 021984, PL 022863, PL 024063	NCPGR[1]
TRI 17029, TRI 18219, TRI 2246, TRI 8310	IPK[4]
Emmer 1	Line from own breeding (University of Warmia and Mazury)
T. spelta	
UWM 10, UWM 11,UWM 12,UWM 13,UWM 14	Lines from own breeding (University of Warmia and Mazury)
PL 021981	NCPGR[1]
TRI 17506, TRI 17513, TRI 3419, TRI 982	IPK[4]
T. aestivum	
Frontana	Own reproduction (University of Warmia and Mazury)
Parabola, Hena, Helia, Eta, Broma, Torka, Bonty, Nawra, Kontesa, Koksa, Jota	PBS[5]

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RESULTS AND DISCUSSION

The spectra produced by the grain of the four analyzed *Triticum* species were characterized by similar absorbance in the majority of wavenumber ranges (Fig. 1). The absolute maximum values for the species (ABS=1.955) corresponded to wavenumber 999.07 cm^{-1}, and local maxima were noted for wavenumbers 2925.85 cm^{-1} (ABS=0.387) and 3 288.25 cm^{-1} (ABS=0.660). Significant variations in absorbance were reported for all species in four wavenumber ranges: 3 616.33-3 651.05, 3 425.39-3 535.32, 2 246.95-3 031.93 and 889.136-1 001.00 cm^{-1}. The above results imply the presence of significant differences in 546 out of 1 997 wavenumbers *ie* in more than 27% cases which can be described as indisputably species-specific.

Wavenumbers differentiating the tested species were analyzed by PCA. Very strong discrimination was achieved in respect of the first two components where 72.16% total variation was explained by PC1 and 22.88% by PC2 to produce a total of 95.04% explained variation. The strongest discrimination was noted with regard to *T. dicoccum, T. monococcum* and *T. aestivum* where PCA identified distinct species-specific clusters. *T. spelta* was marked by a significant range of variability – one of the studied lines (UWM 10) showed considerable similarity to common wheat, whereas lines K18 and K20 were highly similar to einkorn.

Information regarding the use of FTIR/ATR in taxonomic studies is scant. Nie *et al.* (2006) discussed the above method usefulness in taxonomic studies of *Fusarium* fungi. Kos *et al*. (2007) observed that FT-IR/ATR supports quick determinations of deoxynivalenol concentrations in maize, thus indicating that this technique could be applied in breeding programs aimed to develop *Fusarium* head blight resistant genotypes.

CONCLUSIONS

1. The obtained results suggest that Fourier transform infrared – attenuated total reflection is a useful tool for identifying the grain of various hulled wheat species. The studied species differed significantly in the values of absorbance, in particular in the wavenumber ranges for lipids, and for cellulose and hemicellulose.

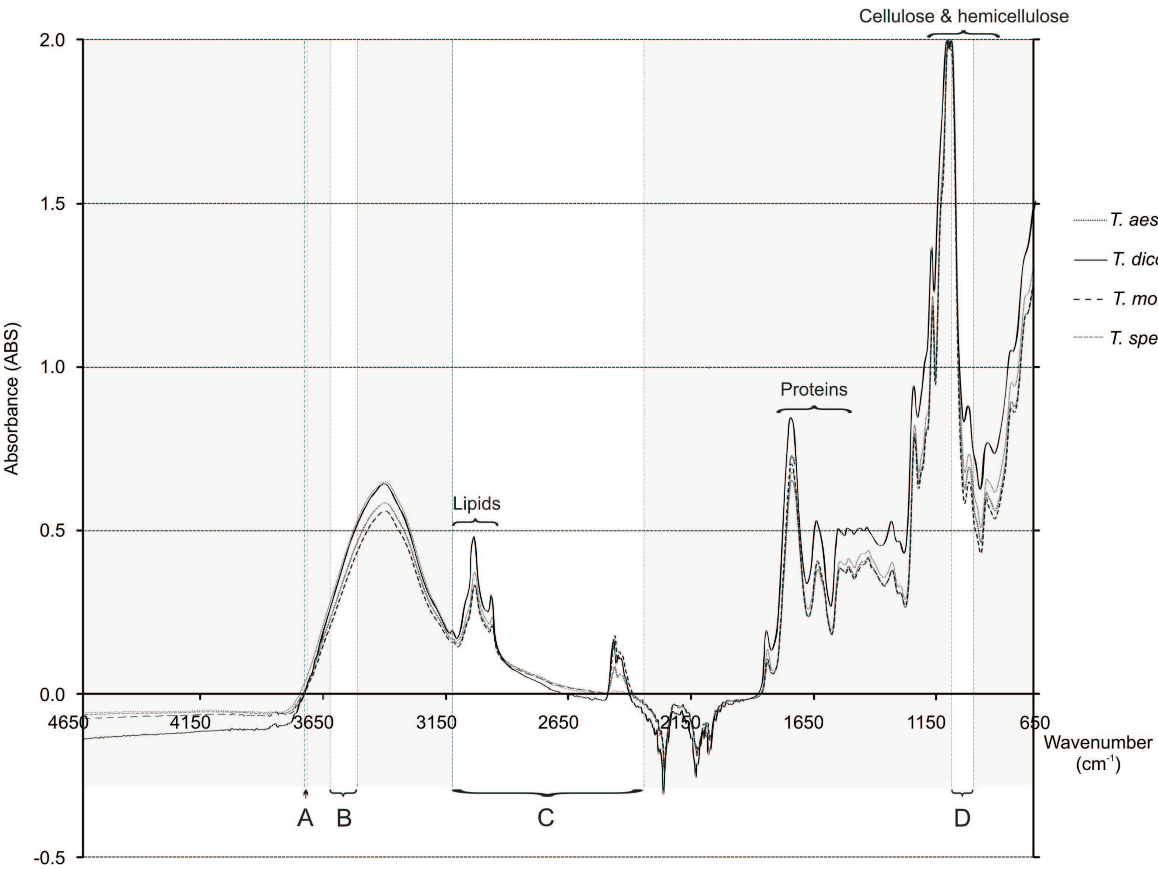

Fig. 1. Average absorbance values produced by FT-IR/ATR for the grain of four *Triticum* sp. species. A, B, C, D – wavenumber ranges showing significant differences between all species. The ranges for lipids, proteins, cellulose and hemicellulose (2 996-2 800, 1 800-1 485, and 1 185-900 cm^{-1}, repectively) follow Nauman *et al*. (2010).

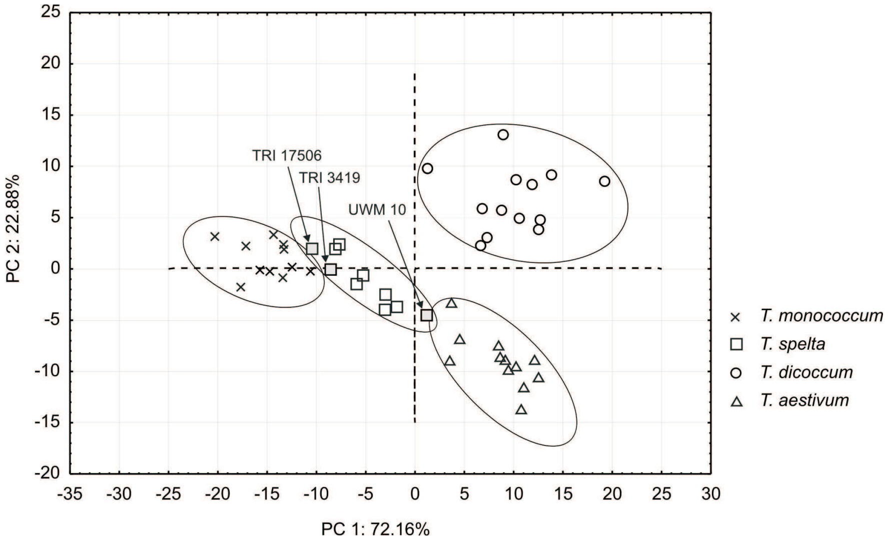

Fig. 2. Principal component analysis of absorbance values for wavenumber ranges differentiating the examined *Triticum* sp. species. Spelt lines showing considerable similarity to the group of *T. monococcum* lines (TRI 17506 and TRI 3419) and *T. aestivum* cultivars (UWM 10) are marked.

2. The applied method can be effectively used in the process of evaluating the quality of consumer grain and seed material.

3. Examination of other *Triticum* genotypes with Fourier transform infrared – attenuated total reflection combined with phytochemical analyses allows to develop a quick method for discrimination of various wheat species.

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