Seed Quality Analysis of Ethiopian Mustard (*Brassica carinata* A. Braun) by Near Infrared Spectroscopy

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INTRODUCTION

Ethiopian mustard (*Brassica carinata* A. Braun) is an oilseed species with high potential as a crop for Mediterranean (semi-arid) conditions and as a genetic source for characters of agronomic importance. In the last 30 years, Near Infra-Red Spectroscopy (NIRS) has been widely used as a rapid and accurate method for qualitative and quantitative analysis in agriculture and the food industry for many years (Williams and Norris, 1987; Osborne et al., 1993). The Department of Agronomy and Plant Breeding of the Institute for Sustainable Agriculture (IAS, CSIC), has been using NIRS for the last fifteen years, to determine the seed quality components in different plant species (De Haro et al., 1989; Velasco et al., 1992; Font et al., 1998, 2000). The most attractive features of analysis using NIRS are its speed, minimal sample preparation and its non-destructive nature thus making it possible to analyse large number of samples in a short time.

The objective of this work is to test the potential of NIRS to determine the oil, protein and acid detergent fiber (ADF) contents of intact seeds of *Brassica carinata* accessions.

MATERIAL AND METHODS

Ten plants from each of the 100 accessions of *B. carinata*, chosen at random from the collection at IAS (Córdoba, Spain) were grown in field plots at Córdoba, Spain, from November 2000 to June 2001.

To perform NIRS calibrations for protein, oil and ADF content, 3g samples of intact seed from 1000 plants (100 accessions x 10 plants/accession), were placed in an NIRS sample holder (3 cm in diameter) and scanned in an NIR spectrophotometer (NIRSystems model 6500, Foss-NIRSystems, Inc., Silver Spring, MD, USA) equipped with a transport module, in the reflectance mode, acquiring their spectra at 2 nm intervals over a wavelength range from 400 to 2500 nm (VIS + NIR regions). On the basis of their spectral features (Mahalanobis distance (*H* statistic) from each sample to the average of the product library), a sub-set of 100 samples for oil and protein, and 41 samples for ADF as representative of the whole spectral variability contained in the entire set, were selected for performing NIR calibrations. Reference analytical values for oil, protein and ADF contents for the selected samples were obtained by Nuclear Magnetic Resonance (NMR), Kjeldahl (AOAC method 979.09, 1990) and the procedure described by Goering and Van Soest (1970), respectively. Using the program GLOBAL v. 1.50 (WINISI II, Infrasoft International, LLC, Port Matilda, PA, USA), different mathematical treatments (0,0,1,1 (derivative, gap, first smooth, second smooth); 1,4,4,1; 2,5,5,2) were used to compute the different calibration equations. Standard error of cross-validation (SECV) obtained from cross-validation was used to test the ability of the equations obtained to predict the different quality parameters, as this statistic is the best single estimate of the prediction capability of an equation (Shenk and Westerhaus, 1996). For each parameter, the equation with the best combination of the statistics ratio of standard deviation (SD) to SECV, and coefficient of determination (1-VR), was selected as the best equation and used for evaluating the ability of NIRS to predict this character on unknown samples belonging to the same plant population.

RESULTS AND DISCUSSION

Second derivative of the raw optical data gave the best combination of 1-VR and SD/SECV ratio for the three characters studied. The selected calibration equations for oil, protein and ADF resulted in standard errors of calibration (SEC) of 1.36, 0.50 and 0.50 % DW, and coefficients of determination in the calibration (R^2) of 0.93, 0.99 and 0.94, respectively (Table 1), which indicate equations with excellent precision (Shenk and Westerhaus, 1996). The equation for protein showed the best fit of the data by the model in the cross-validation, resulting in a SD/SECV ratio of 6.85 (Fig. 1), being this equation useful for quality control (Batten, 1998). On the basis of this ratio, equations for oil (SD/SECV= 3.61) and ADF (SD/SECV= 2.21) (Fig. 2 and 3) could be used for screening purposes. The 1-VR coefficients were over 0.90 for the three equations selected.

A general standard for NIRS determinations of protein and ADF (AOAC method 989.03) has been published by the Association of Official Analytical Chemists, and many authors have been extensively used NIRS as an alternative method for the analysis of oil

(Starr et al., 1985; Tkachuk, 1987; Panford et al., 1988; McGregor, 1990; Williams and Sobering, 1993; Daun et al, 1994), protein (Ribaillier and Maviel, 1984; Starr et al., 1985; Panford et al., 1988; Hartwig and Hurburgh, 1990; Williams and Sobering, 1993; Daun et al, 1994), and ADF (Michalski et al., 1992; Font et al., 2000). These authors reported standard errors of performance (SEPs) for these characters that varied widely depending on factors as sample pretreatment (intact or ground seed) or the spectroscopic technique used (reflectance or transmittance). Thus, SEPs for oil ranged from 0.2% in ground rapeseed (Panford et al., 1988) to about 1% in intact seed (McGregor, 1990; Williams and Sobering, 1993). SEPs reported for predicted protein content ranged from 0.09 % (Panford et al., 1988) to close 1% in many cases. Michalski et al., 1992 reported a SEC of 1.62 % for ADF in rapeseed, and Font et al., 2000 obtained a SECV of 0.96 % in a multi-species calibration of different species of *Brassica*.

Table 1. Calibration and cross-validation statistics (% DW) for oil (n= 100), protein (n=100) and ADF (n= 41) content of *B. carinata* samples used in the calibration equations.

Calibration						**Cross-validation**	
component	range	Mean	SD	SEC	R^2	SD/SECV	1-VR
oil	25.60-54.60	43.12	5.38	1.36	0.93	3.61	0.92
protein	16.80-37.80	24.23	4.66	0.50	0.99	6.85	0.98
ADF	5.33-14.70	9.84	2.10	0.50	0.94	2.21	0.92

CONCLUSIONS

Results reported in this work show that it is possible to use NIRS to determine the oil, protein and ADF contents on intact seed samples of Ethiopian mustard with enough accuracy for screening and plant breeding purposes. The use of this non destructive technique represents an important reduction of the analysis time at a low cost and without using hazardous chemicals.

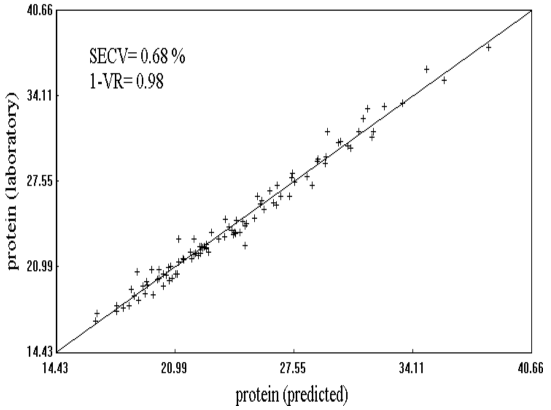

Fig. 1. Cross-validation scatter plot for protein (laboratory vs. predicted)

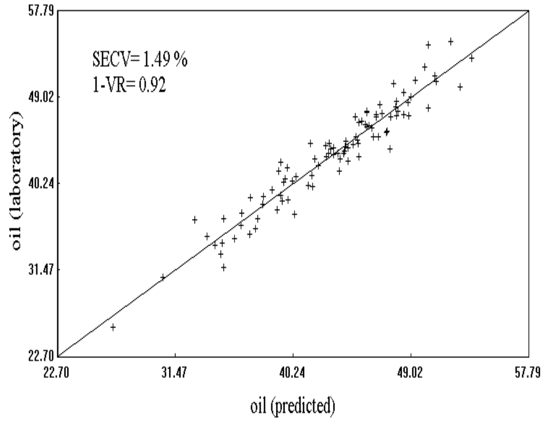

Fig. 2. Cross-validation scatter plot for oil (laboratory vs. predicted)

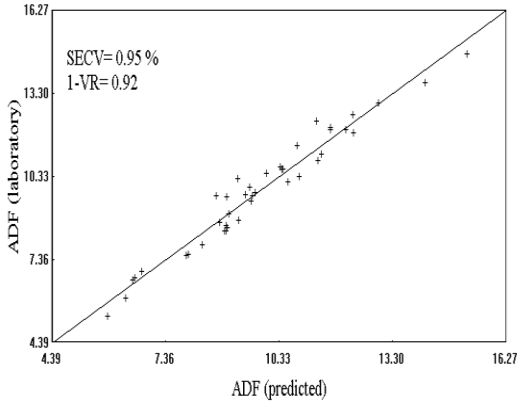

Fig. 3. Cross-validation scatter plot for ADF (laboratory vs. predicted)

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