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Evaluation of Near-Infrared Reflectance Spectroscopy for Predicting Stover Quality Trait in Semi-Exotic Populations of Maize

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Abstract: Near-infrared reflectance spectroscopy (NIRS) analysis was investigated as a means of predicting quality parameters in semi-exotic maize stover. These parameters included crude protein (CP), neutral detergent fibre, acid detergent fibre and *in vitro* dry matter digestibility (IVDMD). Samples of semi-exotic maize stover were collected during three growing seasons (1989, 1990, 1991) from three locations in Catalonia, Spain. Calibration equations were obtained by multiple linear regression of conventional laboratory values on NIRS data from 84 samples and verified with 20 additional samples. Separate NIRS calibration were developed also within year (1989 and 1990, respectively). A Bran + Luebbe InfraAnalyzer model 450 was used for the study. In the multi-year calibration the coefficients of squared multiple correlation (R^2) ranged from 0·81 for IVDMD to 0·92 for CP and the standard errors of calibration (SEC) ranged from 0·35 for CP to 1·46 for IVDMD. The study showed that NIRS analysis can be used to evaluate the quality of semi-exotic maize in breeding programmes.

Key words: near-infrared reflectance spectroscopy, semi-exotic maize stover, crude protein, neutral detergent fibre, acid detergent fibre, *in vitro* dry matter digestibility.

INTRODUCTION

Forage maize is an important feed for ruminants (cattle, sheep, etc) due to it high digestibility of organic matter. Nevertheless, the forage maize crop is a highly heterogeneous crop. Its quality is related to the composition and the physiological status of plants at harvest which depend on the growing conditions (weather, soil, cultural practices, developmental stage and hybrid choice).

Semi-exotic populations of maize with late maturity can be obtained by crossing exotic germplasm with adapted inbred lines. Being very productive, these populations give an appropriate starting point for Mediterranean-type conditions.

Criteria to be considered for the discrimination between semi-exotic populations regarding their production of digestible dry matter were discussed by Casañas *et al* (1991). Although the material is intended for use as forage, maximization of ear yields is still a goal. Selection for high stover digestibility, high cell wall digestibility and low neutral detergent fibre (NDF) are important considerations in a strategy for improving the nutritive value of the plant.

Near-infrared reflectance spectroscopy (NIRS) measurements can be used to predict nutritive quality. The spectrum of reflectance measurements contains indirect non-specific information about the composition of the sample. This information can be extracted using appropriate data treatments (Osborne and Fearn 1986; Goedhart 1990).

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NIRS is capable of rapidly determining various chemical constituents of forages with a high degree of accuracy (Norris *et al* 1976; Shenk *et al* 1979; Marten *et al* 1984). In maize (*Zea mays* L), NIRS has been proposed as a tool for the prediction of the nutritive value of the forage in breeding programmes (Melchinger *et al* 1986; Pinter *et al* 1986; Valdes *et al* 1987a; Zimmer *et al* 1990), because it is rapid, reliable, non-consumptive and economic, needs no chemical reagents, and multiple analyses can be conducted simultaneously.

The objective of this study was to evaluate NIRS for predicting quality traits of semi-exotic populations of maize stover, primarily with regard to its application in plant breeding and animal feeding.

MATERIALS AND METHODS

Sample collection and preparation

The samples used in this study came from semi-exotic evaluation trials. The exotic populations (40) were supplied by CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo), México. The semi-exotic populations were formed by crossing the exotic material with inbreds Mo17 and B73 which are considered to be good representatives of adapted late pure lines. These populations were studied under irrigation using a randomised complete block design with three replications and a density of 66 000 plants ha^{-1}. Plants were harvested (separating the stover from the ear) when the mean grain moisture in the three replications was approximately 400 g kg^{-1}. The field experiment was conducted between 1989 and 1991 and carried out in three different locations of Catalonia, Spain.

An average of 2·0 kg of chopped material drawn at random of each stover sample was dried at 60°C for 48 h and ground through a mill fitted with a 1 mm screen. Samples of about 300 g were catalogued and placed in plastic boxes.

Chemical analysis

Samples of maize stover were analysed in duplicate. Dry matter was determined by drying in a forced-draught over at 103 ± 1°C for 24 h, crude protein (CP) as N × 6·25 by the Kjeldahl procedure on a Kjeltec Auto 1030 Analyzer (Tecator, Sweden), NDF and acid detergent fibre (ADF) by the method of Goering and Van Soest (1970). *In vitro* dry matter digestibility (IVDMD) was determined using the enzymatic method of Aufrère (1982).

NIRS analysis

The NIRS equipment used in this study consisted of a Bran + Luebbe InfraAnalyzer 450 fitted with 19 discrete filters interfaced to an IBM PS2 (model 30) microcomputer. The methodology for the development of NIRS equations has been given elsewhere (Albanell *et al* 1993).

A multiyear calibration was developed using 84 samples from the three years and the three sites, and the validation using 20 samples. Separate NIRS calibration were developed also within year (1989 and 1990, respectively), using 34 samples for the calibration set and 10 for validation set.

Selection was made so that the calibration samples were representative of the range of composition (CP, NDF, ADF, IVDMD) and samples in the validation set were not used in the calibration set or vice versa. Quadruplicate reflectance readings (two sample cups with two different rotating positions) were obtained for all samples used and were averaged before selecting the equations with the best fit for the NIRS predictions of quality parameters.

Filter constants were assessed by using linear regression analysis. The best equation for each constituent was chosen by the optimal combination of the statistics from equation development: high R^2 (squared multiple correlation coefficient), low standard error of calibration (SEC) and high F values in the calibration set. The final step in calibration was validation, which was necessary to obtain an independent measure of the accuracy of the equations, expressed as high r^2 (squared simple correlation coefficient), low standard error of prediction (SEP) and low bias.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of the sample sets used in the study. Chemical analysis indicated that the calibration and validation sets covered similar ranges for each component and the samples of multiyear calibration represented the variability that exists in the predicted parameters.

The optimal calibrations for semi-exotic maize stover samples were obtained using six terms. The results of the calibration and validation are shown in Table 2.

Fourteen wavelengths were used in the equations for 1990 calibration and 15 wavelengths in the equations for 1989 and multiyear calibrations. The 1759 nm and 1778 nm wavelengths were selected for all calibrations and appeared in all equations of NDF (1989, 1990 and $M-Y$). These wavelengths have been previously used in the prediction of ADF, CP and IVDMD in whole-plant corn forage (Valdes *et al* 1990). Osborne and Fearn (1986) reported that the absorption band at 1759 nm was due to the first overtone stretching vibrations of C–H bonds and 1778 nm band to hemicellulose.

Wavelengths used for analysis of IVDMD in equations 1989, 1990 and multiyear, were selected for analysis of IVDMD in whole-plant corn forage by the

TABLE 1
Chemical composition of the semi-exotic maize stover samples used in NIRS analysis (results are expressed as g per 100 g DM)

n	*CP*		*NDF*		*ADF*		*IVDMD*	
	Mean (Range)	*SD*	*Mean (Range)*	*SD*	*Mean(Range)*	*SD*	*Mean (Range)*	*SD*
Cal 89 34	7·7 (6·1–8·9)	0·6	68·9 (61·5–74·0)	2·9	41·1 (34·9–44·5)	2·1	50·2 (41·0–60·1)	3·7
Val 89 10	7·7 (6·3–8·4)	0·6	69·3 (64·8–73·6)	2·5	41·8 (39·7–45·1)	1·8	50·7 (47·8–53·9)	1·7
Cal 90 34	6·5 (4·5–9·3)	1·4	66·5 (61·3–73·3)	3·0	38·5 (33·0–45·9)	2·9	48·3 (40·6–55·2)	3·4
Val 90 10	5·7 (5·0–6·4)	0·4	66·4 (64·3–69·4)	1·8	39·1 (36·9–41·0)	1·2	47·7 (45·4–49·6)	1·4
Cal M-Y 84	7·1 (4·5–9·3)	1·2	67·8 (61·3–74·0)	3·1	39·8 (33·0–45·9)	2·8	49·2 (40·6–60·1)	3·5
Val M-Y 20	7·0 (4·6–8·8)	1·2	66·7 (61·3–73·3)	2·7	38·0 (31·5–42·9)	2·5	49·8 (41·8–56·6)	3·8

Tilley and Terry method (Valdes *et al* 1987b) and by the pepsin-cellulase technique (Valdes *et al* 1990). Clark and Lamb (1991) observed the frequency distribution of wavelength selection in studies on legumes, grasses and mixtures to measure digestibility (*in vitro* or *in vivo*) and the predominant wavelengths were 1600–1900 and 2200–2300 nm. Even though the 1445 nm wavelength was not the most important, it was common in the three equations of IVDMD and it is attributed to the first overtone of the stretching vibration of a free OH group (Osborne and Fearn 1986).

The values for the coefficients of squared multiple correlation (Table 2) were in general satisfactory. The values obtained in multiyear calibration were 0·92 for CP, 0·90 for NDF, 0·90 for ADF and 0·81 for IVDMD. The values obtained here were comparable with those reported by Melchinger *et al* (1986) and Zimmer *et al* (1990).

The highest correlation of 0·96 was obtained for the CP and ADF for the 1990 calibration. Chemical assays were used to determine CP, NDF and ADF whereas *in vitro* bioassay was used for IVDMD. Generally, a higher precision is associated with chemical analysis than with bioassay.

The SEC values ranged from 0·27 to 1·38 for the 1989 calibration, 0·32 to 0·89 for the 1990 calibration and

TABLE 2
NIRS calibration statistics for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and *in vitro* dry matter digestibility (IVDMD) in semi-exotic maize stover samples

Component	R^{2a}	*SEC*[b]	*Wavelength*[c]					
			1	*2*	*3*	*4*	*5*	*6*
CP								
1989	0·81	0·27	1734	1778	2180	2310	1818	1982
1990	0·96	0·32	1778	1734	2208	2270	2348	2336
M-Y	0·92	0·35	1759	1734	2270	2208	1982	1940
NDF								
1989	0·94	0·82	1759	1778	2310	2190	2100	1445
1990	0·93	0·90	1759	1722	1778	2270	2336	1818
M-Y	0·90	1·01	1778	1759	1722	2230	2208	1680
ADF								
1989	0·90	0·72	2348	2310	2100	2139	1445	1940
1990	0·96	0·65	1759	1778	1982	2139	2208	1680
M-Y	0·90	0·87	1759	1818	1982	2139	2100	1445
IVDMD								
1989	0·88	1·38	1778	1818	2270	1445	2336	2348
1990	0·94	0·89	1759	1818	2270	1722	2310	1445
M-Y	0·81	1·56	1818	1759	1722	2270	1445	2336

[a] R^2, squared multiple correlation coefficient.
[b] SEC, standard error of calibration.
[c] Filters in order of importance according to the size of the partial F value.

0·35 to 1·56 for the multiyear calibration (Table 2), being the highest for IVDMD and lowest for CP. The SEP was comparable to SEC in most cases.

The NIRS technique has been used in maize to determine dry matter digestibility and cell wall constituents: CP, and ADF of stover by Melchinger *et al* (1986); ADF, CP and IVDMD determined by a two-stage pepsin cellulase technique of whole plant by Valdes *et al* (1990) and NDF, and ADF, of stover by Zimmer *et al* (1990).

The values for the squared multiple correlation coefficients (R^2) were comparable or better in the present study than those reported by Melchinger *et al* (1986) and Valdes *et al* (1990). The SEC estimates varied in these studies and the present estimates were, however, comparable or better in most cases.

The statistical results from linear regression analysis comparing the actual results of chemical analysis with those predicted from NIRS analysis are shown in Table 3. The squared simple correlation coefficients obtained were as follows: CP (0·81 and 0·89), NDF (0·86 and 0·85), ADF (0·81 and 0·72) and IVDMD (0·88 and 0·86) in 1989 and 1990 calibrations, respectively. The r^2 values obtained with the multiyear calibrations were 0·88, 0·85, 0·90 and 0·88 for CP, NDF, ADF and IVDMD, respectively.

The SEP values ranged from 0·24 to 1·88. Except for CP (in multiyear calibration) and ADF (in 1990 calibration), the prediction errors were higher than the calibrations errors. Melchinger *et al* (1986) and Zimmer *et al* (1990) also obtained in maize stover SEP comparables to SEC or even smaller than the SEC in some parameters.

The standard error, slope and bias values of selected in multiyear equations indicated that CP, NDF, ADF were estimated acceptably in semi-exotic maize stover samples. The IVDMD (by the enzymatic method) was well predicted by the 1990 calibration than the multiyear calibration.

The results obtained in this study showed sufficient accuracy in the predictions of chemical composition of semi-exotic maize stover, for preliminary selection in our plant breeding programme.

TABLE 3
Validation statistics for NIRs determination of chemical composition of semi-exotic maize stover

Component	r^{2a}	*SEP*[b]	*Bias*	*Slope*
CP				
1989	0·81	0·31	−0·04	1·01
1990	0·89	0·24	0·04	0·83
M-Y	0·88	0·44	0·21	1·11
NDF				
1989	0·86	1·07	0·56	0·86
1990	0·85	1·16	−0·51	0·68
M-Y	0·85	1·07	−0·42	0·82
ADF				
1989	0·81	1·14	0·31	0·81
1990	0·72	0·99	0·11	0·78
M-Y	0·90	0·82	−0·09	0·88
IVDMD				
1989	0·88	1·88	−0·40	0·63
1990	0·86	1·32	0·04	0·91
M-Y	0·88	1·64	−0·40	1·04

[a] r^2, squared simple correlation coefficient.
[b] SEP, standard error of prediction.
[c] Bias, difference between chemical and NIRS values.

CONCLUSIONS

In conclusion, our results indicate that the NIRS technique can be used to evaluate the quality of semi-exotic maize in breeding programmes, using a multi-year calibration with maize stover samples drawn from several sites and three harvesting seasons. However, recalibration will be necessary, especially for IVDMD prediction, over the next few years.

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