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Predicting the oil contents in sunflower genotype seeds using near-infrared reflectance (NIR) spectroscopy

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ABSTRACT. The aim of this experiment was to calibrate the NIR spectroscopy equation to evaluate the oil content of sunflower seeds from different genotypes produced under different environmental conditions in Brazil. The spectra of 901 standard samples obtained from 88 hybrids and 116 lines, which were evaluated in 11 locations, were collected from intact seeds (achenes) and correlated with data generated by nuclear magnetic resonance analysis. The calibration was determined by linear regression using partial least squares to estimate the parameters. The goodness of fit was evaluated using the coefficient of determination (R^2), standard error of calibration (SEC) and the standard error of performance (SEP). The wavelengths ranging from 1319 to 1760 nm were selected for the calibration. The R^2 was 0.87, the SEC was 2.39, and the SEP was 1.97. The oil content values obtained for the 19 hybrid seeds analyzed by NIR spectroscopy that were not included in the calibration were similar to the values obtained using the chemical method. The similarities between the values obtained using both methods and the R^2, SEC and SEP values indicated that it is possible to establish a calibration equation using NIR spectroscopy to determine the oil contents of sunflower seeds produced under Brazilian field conditions.

Keywords: *Helianthus annuus*, intact seed, spectral analysis.

Predição do teor de óleo em sementes de genótipos de girassol por espectroscopia no infravermelho próximo (NIR)

RESUMO. O objetivo desse trabalho foi estabelecer uma equação de calibração pela análise da espectroscopia de NIR para a avaliação dos teores de óleo em sementes de genótipos de girassol obtidas em condições ambientais brasileiras. Os espectros de 901 amostras padrões, provenientes de 88 híbridos e 116 linhagens avaliados em 11 localidades, foram coletados de aquênios intactos e correlacionados com os valores de óleo obtidos por análise de ressonância magnética nuclear. A equação de calibração foi determinada por regressão linear, cujos parâmetros foram estimados pelo método de mínimos quadrados parciais. A precisão foi verificada pelo coeficiente de determinação (R^2), erro padrão de calibração (SEC) e pelo erro padrão de desempenho (SEP). A região de comprimento de onda entre 1319 e 1760 nm foi selecionada para a calibração. O R^2 foi 0,87, o SEC foi 2,39 e o SEP foi de 1,97. Adicionalmente, a análise do teor de óleo pelo NIR de 19 híbridos não incluídos na calibração foi similar aos valores obtidos no método químico padrão. Esta similaridade e os valores de R^2, SEC e SEP possibilitaram inferir que é possível estabelecer uma equação de calibração pela espectroscopia de NIR para a avaliação do teor de óleo em sementes de genótipos de girassol cultivados em condições ambientais brasileiras.

Palavras-chave: *Helianthus annuus*, sementes intactas, análise spectral.

Introduction

Sunflower (*Helianthus annuus* L.) ranked fourth in production in the World Ranking in 2011 with a world production of 12.21 million of metric tons (USDA, 2012). In Brazilian cultivars, the seed oil contents range from 38 to 48%, and the national agriculture policy paid bonuses to sunflower growers who cultivated varieties with seed oil contents above 40% (GRUNVALD et al., 2008; PORTO et al., 2008). Currently, the Sunflower Breeding Program is attempting to develop cultivars with high seed oil contents.

The oil contents in sunflower seeds can be chemically determined using extraction with n-hexane or ether as solvents in Soxhlet apparatuses (IAL, 2008).These solvents, which are hazardous to human health, also to the environment. Although this protocol

is methodologically efficient, the process destroys the seeds, which demands more time and cost for each analysis. The chemical extraction protocol is inapplicable when a large number of samples or a small quantity of seeds produced by specific breeding tests need to be analyzed before they can be planted in the next cycle of plant selection (ROBERTSON; BARTON, 1984). An alternative method to the traditional chemical extraction method uses nuclear magnetic resonance (NMR) (COLLINS et al., 1967; ROBERTSON; BARTON, 1984). The NMR method is fast, reliable and does not destroy the sample, but the samples must be predried before the analysis (MADSEN, 1976).

Near-infrared reflectance (NIR) spectroscopy has been applied to several species of plants with similar purposes because it is fast, effective, nondestructive and does not require predrying (SATO et al. 1991; BATTEN, 1998; MATTHÄUS; BRUHL, 2001). NIR spectroscopy also allows the simultaneous analyses of oil, protein, fiber and many other components (BATTEN, 1998). The efficacy of this technique to determine the chemical composition of sunflower seeds was reported by Robertson and Barton (1984), Pérez-Vich et al. (1998), Sato (2002), Fassio and Cozzolino (2004), Velasco et al. (2004) and Biskupek-Korell and Moschner (2006).The application of this method, however, requires that the equipment is calibrated to correlate with the NIR spectral information from a reference method. Standard samples representing all the expected variations in the contents must be used to construct a reference data set (VELASCO et al., 1998). In addition to the plant species, the environmental conditions should also be considered because they can interfere with the spectral absorbance (BATTEN, 1998; FASSIO; COZZOLINO, 2004; SAARONI et al. 2010). The equations currently available and necessary to apply the NIR method to the sunflower seeds were fit to data collected in many other countries that have distinct field conditions toward Brazil.

The objective of the current research was to calibrate an equation to allow the use of NIR spectroscopy to determine the oil contents of sunflower seeds produced under the field conditions of Brazil.

Material and methods

The oil content data from 901 standard samples of sunflower seeds collected from 88 hybrids and 116 lines were used to calibrate the NIR equipment. Hybrid seeds with oil contents ranging from 35 to 52% were produced in field trials established in randomized complete blocks with four replicates. Following the regional recommendations, some field trials were sowed in February and March while others were sowed in August and October. The field trials were conducted in 11 different locations from 2007 to 2009 (Table 1). These locations are part of The Sunflower National Trials under the coordination of the Embrapa Soybean Research Center and with participation of various governmental and private institutions. The standard lines from the Sunflower Germplasm Seed Bank (BAG) of the Embrapa Soybean Center with seed oil contents ranging from 24 to 38 % were sowed only in the agriculture fields of the Londrina County (Paraná State, Brazil) where the sunflower breeding program is under development.

The referential method applied to determine the seed oil contents from the standard samples used nuclear magnetic resonance (NMR) (OXFORD 4000, OXFORD ANALYTICAL INSTRUMENTS Ltd.). The spectra from these samples were collected from the intact sunflower seeds (with seed coat) of approximately 200 achenes placed in the cup spinner accessory combined with the integrating sphere modulus of the NIR equipment (Model Antaris II, Thermo Scientific). The absorbance spectra (Log 1 R^{-1}) were obtained in the wavelength range from 1100 to 2500 nm. Every spectrum represented the average from 32 scans with a resolution of 4 cm^{-1}.

Table 1. Regional characteristics of the Sunflower National Trials from 2007 to 2009.

Location	Growing season	Latitude	Longitude	Altitude	Soil classification
Roda Velha (BA)[1/]	2007/2008[3/]	12°07' S	45°83' W	755 m	Red-yellow latosol
Planaltina (DF)[1/]	2008[4/]	15°35'30" S	47°42'30" W	1007 m	Dystrophic red latosol
Porangatu (GO)[1/]	2009[4/]	13°18'29" S	49°06'27" W	357 m	Red latosol
Jaguariúna (SP)[1/]	2007/2008[3/]	22°42'20" S	46°59'09" W	584 m	Red-dark latosol
Muzambinho (MG)[2/]	2008 [4/]	21°22'33" S	46°31'32" W	1048 m	Red latosol
Leme do Prado (MG)[2/]	2007/2008[3/]	17°03' S	42°48' W	812 m	Dystrophic red latosol
São José dos Quatro Marcos (MT)[2/]	2008[4/]	15°39'11" S	58°16'56" W	280 m	Eutrophic red-yellow argisol
Vilhena (RO)[2/]	2008[4/]	12°47'12" S	60°03'39" W	600 m	Dystrophic red-yellow latosol
Londrina (PR)[1/]	2008[4/]	23°18'37" S	51°09'46" W	566 m	Dystroferric red latosol
Xaxim (SC)[1/]	2007/2008[3/]	26°57'20" S	52°30'34" W	836 m	Red latosol
Passo Fundo (RS)[1/]	2008/2009[3/]	28°07'38" S	52°17'46" W	21 m	Dystroferric red latosol

[1/] Final trial of the first year; [2/] Final trial of the second year; [3/] Seed sowing in August and October;[4/] Seed sowing in February and March.

The equation was fit using linear regression with the parameters estimated by partial least squares (PLS) using the TQ Analyst software.

Norris smoothing filter. Segments of 3 nm and their intervals of 12 nm were used in this correction.

The goodness of fit was determined using both the coefficient of determination (R^2) and the standard error of the calibration (SEC). The performance of the equation was evaluated by the standard error of performance (SEP) using 34 random samples taken from all the original 901 standard samples.

Furthermore, the seed oil contents from 19 hybrids evaluated using the calibrated equation with NIR spectroscopy were compared to the seed oil contents obtained with the Soxhlet apparatus using n-hexane as the solvent (IAL, 2008). These hybrids, which were not included in the calibration protocol, were evaluated under field conditions in Paracatu County (MG) in 2010 under randomized complete blocks in a split-plot arrangement of treatments with three replications. The main plots represented the genotypes, and the subplots represented the methods of seed oil evaluation. These data were evaluated using ANOVA with the Genes software (CRUZ, 2006).

Results and discussion

The absorbance maxima were determined in the spectral range from 1319 to 2500 nm (Figure 1), but only the range from 1319 to 1760 nm was used to calibrate the equation. The coefficient of determination (R^2) in this wavelength range was 0.87, the standard error of the calibration (SEC) was 2.39, and the standard error of performance (SEP) was 1.97 (Figure 2). Although the highest absorption peaks were observed in the wavelength range of 1864 to 2500 nm, this range was not used in the calibration because the equation precision would reduce to a level in which the R^2 = 0.83, SEC = 2.79 and SEP = 2.57. The reduction can be attributed to absorbance at a wavelength of 1930 nm, which is associated with the O-H chemical bonds related to water rather than oil contents (FASSIO; COZZOLINO, 2004).

The current precision (R^2 = 0.87, SEC = 2.39 and SEP = 1.97) was similar to the results reported by Robertson and Barton (1984) who also analyzed the oil contents of intact sunflower seeds using NIR spectroscopy. The precision was lower than the responses reported by Pérez-Vich et al. (1998) who evaluated husked seeds. Robertson and Barton (1984) also stated that the seed coat color can reduce the precision of the equation. The oil content of intact seeds can be determined in a large number of samples during the breeding program to reduce the labor and the time necessary to eliminate the seed coat.

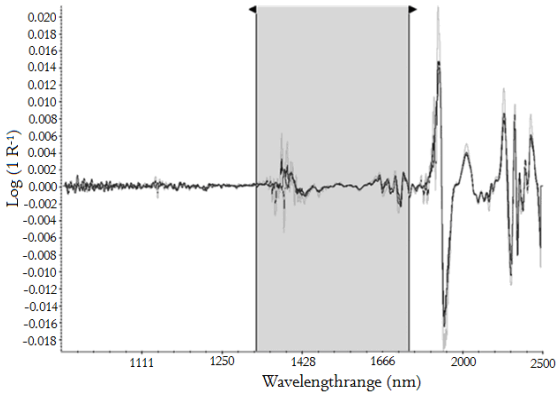

Figure 1. Wavelength range from 1319 to 1760 nm used to calibrate the NIR equation and evaluate the seed oil content of the sunflower genotypes

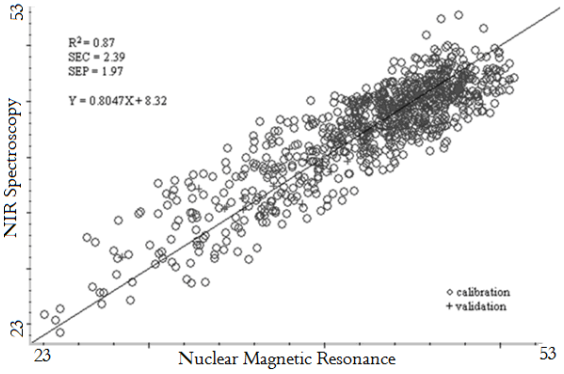

Figure 2. The calibration of the NIR equation to determine the seed oil content of the sunflower genotypes

The variability in the oil content of seed samples can also reduce the precision of the equation (SHENK; WESTERHAUS, 1993). In this phase of the experiment, the use of NMR rather than the standard chemical method was necessary because we evaluated a large number of samples to calibrate the equation. The results reported in Figure 2 were determined using the standard samples of *Helianthus annuus* with seed oil contents ranging from 24 to 52% (FICK, 1983).

The responses from the hybrids in the Sunflower National Trials used as the standards to calibrate the equation allowed us to detect, apart from the samples with seed oil contents ranging from 35 to 52%, diversity levels in the seed coat colours as well as a representation of genotypes cultivated under different environmental conditions

in Brazil. Furthermore, the standard lines stored in the active germplasm bank (BAG) of the Embrapa Soybean Research Center with seed oil contents from 24 to 38% improved the representation of the variability in the oil contents that existed in this plant species. Thus, the environmental and genotypic effects were considered in this step of the protocol calibration. Although the samples were only collected in Londrina County (Northwestern Paraná State, Brazil), the range of oil contents will be useful to select genotypes in the first stages of the breeding program.

The precision equation evaluated using the SEP was compared with the seed oil content evaluated using the standard chemical analysis in which the samples were obtained from a field experiment under randomized complete blocks. Differences between the methods and the interaction methods x hybrids were not significant (Table 2). These responses indicated similarity among the methods.

Table 2. Analysis of variance for data of the oil seed contents of sunflower hybrids evaluated by NIR spectroscopy and chemical extraction.

Source of Variation	Degree of freedom	Mean square
Block	2	3.2153
Hybrids	18	50.1853**
Error (a)	36	2.77
Methods	1	4.9479ns
Hybrids x Methods	18	3.8147ns
Error (b)	38	3.28
Mean	44.74	-
C.V. (%)1/ plot	3.72	-
C.V. (%) subplot	4.05	-

**significant at 1% by the F test; ns nonsignificant; 1/coefficient of variation.

In the current experiment, the NIR calibration was based on standard samples from different Brazilian States representing different edaphoclimatic conditions (Table 1). The average seed oil content results obtained using the NIR spectroscopy experimental design were similar to the results obtained using the chemical method. This comparison and the R^2, SEC and SEP values indicated that the evaluation of intact seeds using the calibration equation estimated using NIR spectroscopy was suitable to discriminate the seed oil contents for different sunflower genotypes cultivated in Brazil.

Conclusion

It was possible to establish a calibration equation using NIR spectroscopy to determine the oil contents of sunflower seeds produced under Brazilian field conditions.

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