PREDICTION OF SEED COAT PROPORTION IN LUPINS BY NEAR INFRARED REFLECTANCE SPECTROSCOPY: PRELIMINARY RESULTS

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ABSTRACT

Compared with other legumes, lupins have a high percent of seed weight in the seed coat. It has been shown that genetic variation exists for seed coat proportion in lupin species and that the prospect for improvement by breeding is good. However, the standard method for assessing seed coat thickness involves manual separation of seed fractions, drying and weighing. This is laborious, time-consuming, and it destroys the seeds.

Near infrared reflectance spectroscopy (NIRS) was evaluated as a non-destructive and quick means for predicting the proportion of seed coat in lupins. A preliminary calibration was developed based on 70 seed samples comprising *Lupinus angustifolius*, *L. albus* and *L. luteus*. Spectra were collected in reflectance on whole seeds and percentage of seed coat, obtained by the standard method (mean: 24.27 ± 2.97%) was used as reference data. Several equations, obtained after different mathematical treatment of the spectral data, were compared according to the coefficient of determination and the standard error of a cross validation (R2CV and SECV). The best equation (R2CV: 0.94; SECV: 0.73%) showed a remarkable potential of the NIRS technique for selecting lupin individuals with lower seed coat, indicating that this could be a valuable tool in breeding programs oriented to improve the feeding quality of lupin grains.

KEY WORDS

Lupinus, NIR, hull proportion, grain legumes

INTRODUCTION

Lupin grain is considered as a valuable ingredient in salmon diets and other monogastric animals. However, the high seed coat proportion of lupins compared with other legume grains lowers its nutritional value, because the seed coat has a high fraction of structural carbohydrates and a low protein and oil content. A thicker coat reduces protein and oil content of the grain and, therefore its dry matter and energy digestibility.

The heritability values for seed coat proportion and pod wall thickness, are estimated to be strong, therefore, breeding to reduce these 'primitive' characteristics has been encouraged (Clements *et al.* 2002; Mera *et al.* 2004). In a breeding program oriented to improve the nutritional quality by reducing hull thickness, it is necessary to employ a screening method that is accurate and can prevent the destruction of the seed as a reproductive organ, in order to make use of the best ranked individuals as parent lines. Near infrared spectroscopy (NIRS) has demonstrated such potential and has been used in a number of applications including the food industry (Osborne *et al.* 1993). It can be applied on intact samples and it may be a fast and accurate technique, provided that proper regression equations are developed, on a broad and representative set of calibration samples with dependable reference methods (Alomar and Fuchslocher, 1998).

The objective of this work was to obtain preliminary equations to predict the percentage of seed coat in samples of intact lupin seeds in order to use this method as a tool for screening purposes in breeding programs.

MATERIALS AND METHODS

A set of 70 samples of lupin seeds of *Lupinus angustifolius*, *L. albus* and *L. Luteus* was used to develop a preliminary calibration. Spectra were collected in reflectance with a Foss-Tecator 6500 scanning monochromator (Silver Springs, MD, USA), controlled by a personal computer and the software WINISI II from Infrasoft International (ISI, Port Matilda, PA, USA). A spinning module was used, in which a ring cell was inserted along with the sample, in this case a number of whole seeds. Optical data were transformed to microabsorbance units (log 1/R) and stored in suitable files.

To determine seed coat proportion, dry seeds were allowed to absorb distilled water for 1 h and 20 random seeds per sample were processed, discarding those that did not imbibe. The hull was removed manually, retaining the embryo with the cotyledons. The two fractions were dried at 65°C for 12 h and weighed promptly. Seed coat proportion was reported as a percentage relative to the weight of the whole seeds. These percentages were used as the reference data for calibration.

IN J.A. Palta and J.B. Berger (eds). 2008. 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, 14-18 Sept. 2008, Fremantle, Western Australia. International Lupin Association, Canterbury, New Zealand. ISBN 0-86476-153-8.

Calibration development was performed by the same software described above. Prediction equations were obtained by linear regression and the method chosen was the modified partial least squares (MPLS). Different mathematical treatments of the spectra were tested to extract relevant information (derivative number, subtraction gap, smoothing interval), with or without applying Standard Normal Variance (SNV) and Detrend for scatter correction. SNV scales each spectrum so that the standard deviation is 1.0 to help reduce particle size effects and Detrend removes the linear and quadratic curvature of each spectrum (Fernández-Cabanás *et al*. 2008).

A cross validation method was performed dividing the set of calibration samples into four groups, leaving one group out which was used as a set for prediction, by an equation adjusted with the other three groups of samples. The process was repeated cyclically with all groups and statistical terms of certainty and uncertainty computed at each step and averaged once all the groups of samples had been predicted. This process was also useful to limit the number of terms of the equation in order to avoid overfitting. The best equation was selected according to the standard error (lowest) of cross validation (SECV) and the coefficient of determination (highest) of the cross validation (R2CV). Two passes of elimination of outlier samples were applied. A critical T value of 2.5 was set for 'T' outliers, i.e. samples with abnormally high residuals of predicted versus reference values.

RESULTS AND DISCUSSION

The samples used as calibration set (n=70) presented a mean hull content of 24.3 ± 2.97%, over a 10% coefficient of variation, with a range of 15.97 to 27.42% which represents a valuable variability for breeding purposes and also for developing a NIRS calibration.

The equation selected included eight terms and was obtained with a mathematical treatment of the spectra of 2,8,8; that is, including a second substruction (derivative) order at intervals of 8 data points, with a first smooth in a segment of 8 data points. SNV and Detrend was also applied to the spectral data in this equation. Three samples were eliminated as 'T' outliers; therefore the equation was finally constructed with the data from 67 samples.

The best equation showed an excellent potential of the technique, with a R2CV of 0.94 and a SECV of 0.73%. This value represents about 1/4 of the standard deviation of the reference data (2.97%) and 3% of the average value (24.3%) of hull percentage of the seeds. Calibration is considered adequate for screening purposes if the ratio of SECV to the standard deviation of the reference data (SD/SECV) is between 2.5 and 3.0 (Sinnaeve *et al*. 1997; Flinn *et al*. 1998). It was over 4 in this case.

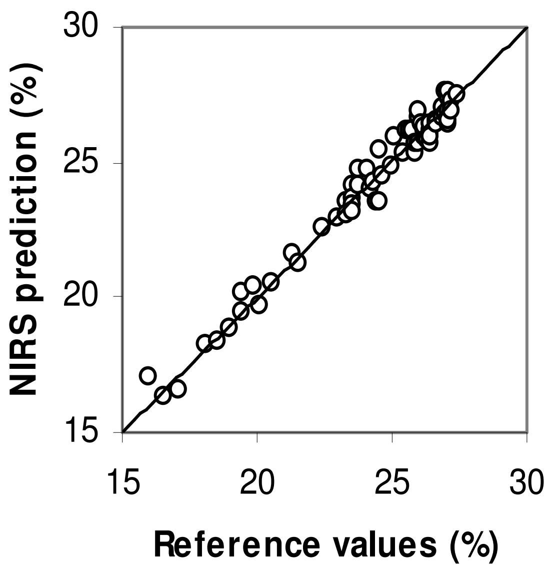

Fig. 1. Reference and NIRS predicted values for seed coat proportion of samples of three lupin species (*Lupinus angustifolius*, *L. albus*, *L. luteus*).

Fig. 1 depicts reference and predicted values As it can be seen, samples tended to be placed with a small deviation and close to the equal response line. Although a higher number of samples were placed near or above the mean value (24%), a few (10) had values at or below 20% of hull content. These samples represent a valuable material for genetic improvement.

In conclusion, NIRS showed a high potential to be applied for screening lupin seeds to be used as parent lines in a program oriented to reduce the seed coat percentage and, consequently, to improve the nutritional value of lupin seeds. Further work is considering a higher number of lines within each lupin species, and hopefully with a broader span in the variable evaluated, especially in the lower segment of values. A different, perhaps more straightforward approach to evaluate hull content, i.e. hull thickness, may also be considered in the future, since seed coat proportion is affected by seed size.

ACKNOWLEDGMENTS

Funding for this research was received from CONICYT Chile through FONDECYT Project 1070232.

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