NIRS prediction of seed coat in intact yellow (*Lupinus luteus*) and narrow-leafed (*L. angustifolius*) lupin grains

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Introduction

Lupin grain is a valuable feed for different animal species, but its high hull content (seed coat) compared to other legumes, reduces the digestibility and feeding value, particularly for monogastric species.[1,2] Variability and heritability of the seed coat has encouraged plant breeders to work on the development of lupin cultivars with a lower hull content.[3]

Breeding programs require fast, accurate and non-destructive methods to select seeds (grains), since many genotypes need to be tested, and the best grains must be used as seeds to develop improved lines. Near Infrared (NIR) Reflectance Spectroscopy appears as a suitable method for this purpose, as it has been successfully employed in a wide range of applications.[4]

This study evaluated NIR spectroscopy as a method to predict seed coat in whole yellow (*Lupinus luteus*) and narrow-leafed (*L. angustifolius*) lupin grains, taking into account that seeds should not be harmed and that few grains from each individual plant are usually available.

Materials and methods

Spectra

Samples of whole grains (*ca*. 70 seeds, 9 g each sample) of *L. luteus* (n = 180) and *L. angustifolius* (n = 445) including different lines and locations, were scanned in the Vis-NIR region (NIRSystems 6500 monochromator, software WINISI II). Samples were placed in ring cells and inserted in a spinning module for the readings.

Reference data

Seeds were soaked in water, coats removed manually and the resulting fractions (hulls, cotyledons) dried overnight in an oven at 65 ºC and weighed to report seed coat percentage, which was used for calibration.

Calibrations

Equations were obtained by modified partial least squares (MPLS), testing different math treatments of the spectra (derivative order, subtraction interval, smooth segment), with or without Standard Normal Variate (SNV) and Detrend.

Equations were tested by cross validation. A critical value of 2.5 was set for "T" outliers (predicted vs. reference residuals) and two elimination passes were performed.

Best equation was selected by root mean standard error (lowest) of cross validation (*RMSECV*), by the proportion of variance explained by the equation (1-*VR*) and the relation between *SD* and *RMSECV* (*RPD*).

Results and discussion

"Raw" spectra (Figure 1) show ample variability, especially in the visible region as a result of different colours and colour patterns in the seed coats. Several absorption bands are present in the NIR region representing bonds from protein, carbohydrates and lipids.

The best equation was obtained with 13 PLS terms, a math treatment of the spectra of 2, 8, 8: second subtraction order (second derivative) across an interval of eight data points (16 nm) and a smooth segment of 8 data points; plus SNV and Detrend. Calibration statistics are presented in Table 1.

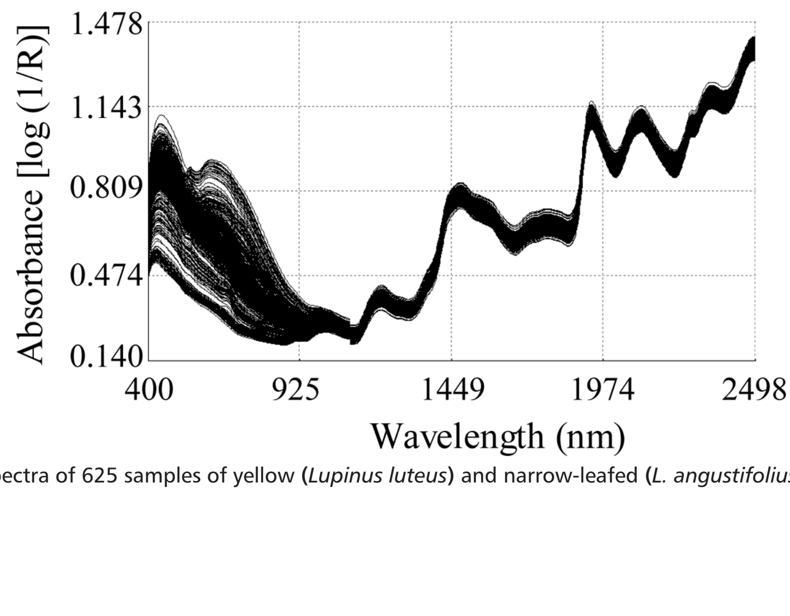

Figure 1. Spectra of 625 samples of yellow (*Lupinus luteus*) and narrow-leafed (*L. angustifolius*) intact lupin grains.

Table 1. Statistics for best calibration.

Parameters for the best equation								
RSQ	*SEC* (%)	*RMSECV* (%)	1-*VR*	Mean (%)	*SD* (%)	Range (%)	*RPD*	*CV* (%)
0.896	0.499	0.556	0.871	22.64	1.54	18.36–26.80	2.77	2.46

This calibration model showed a strong relationship between spectra and percentage of seed coat and, although the RPD value that were lower than 3 for this equation suggests that prediction accuracy could be compromised,[5] it could be used for screening purposes, *i.e.*, ranking lines, varieties or crosses, according to seed coat percentage. In this way, seeds from individuals that present a lower seed coat % can be selected to obtain improved genetic lines. Besides, the prediction error is of a low magnitude, if expressed as coefficient of variation (*CV*) *i.e.*, as the percentage of the average seed coat value (<2.5%). Furthermore, *RER* ("Range Error Ratio") another expression relating *RMSECV*, in this case to the range of the reference values, results in a value >10, which is encouraging for prediction purposes.[6]

Reference versus NIR predicted values are presented in Figure 2.

Samples are well distributed along the equal response (y = x) line. Most samples with lower seed coat percentage belong to narrow-leafed lupins. This results from the fact that a mutant with a low seed coat is present as a parent line in all the samples of this species. Sources of error could be associated with the spectra collection method (whole grains in small ring cells, which presents a fairly small number of seeds to the instrument), variability in the reference method, and the fact that the percentage of seed coat is affected, not only by hull thickness but also by seed size.

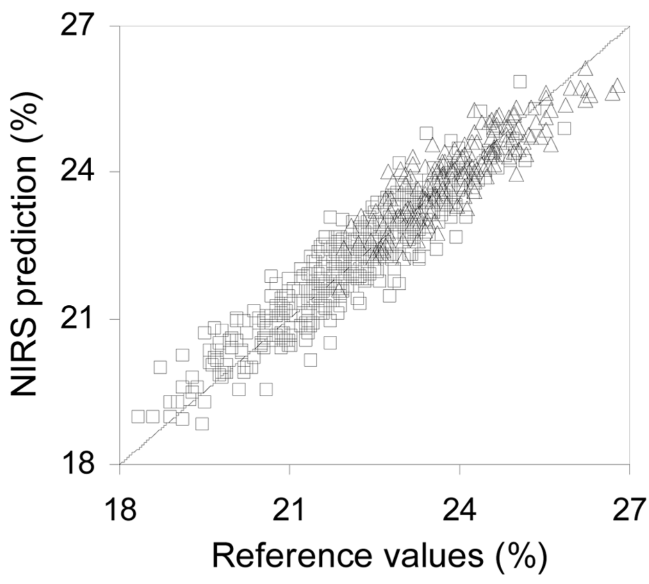

Figure 2. Reference vs. NIRS predicted seed coat percentage of yellow (Δ) and narrow-leafed (□) lupin grains.

Conclusions

NIR spectroscopy can be employed as a fast and useful method for ranking intact lupin seeds according to seed coat (hull content) which is a significant issue in plant breeding programs oriented to improve the feeding value of the crop. This is particularly the case, when many cultivars or crosses from more than one species need to be evaluated.

References

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