Identification of lupin species, varieties and crosses by near infrared analysis of whole seeds

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Introduction

Lupin grains of different species (*Lupinus albus, L. angustifolius* and *L. luteus*) have become a valuable source of nutrients for animals and humans, because of their high content and quality of proteins and their low level of anti-nutritional factors, in comparison to other legumes.[1] Breeding programs are being carried out in different parts of the world to obtain varieties with improved nutritional quality. White lupin (*L. albus*) has distinct, larger and flattened seeds, but the seeds of yellow (*L. luteus*) and narrow-leafed (*L. angustifolius*) lupin are smaller and more difficult to differentiate, particularly because many cultivars or genetic lines, with a variety of colours, colour patterns and colour sizes, have been developed.

Work has been conducted to evaluate different traits on whole seeds by NIR spectroscopy,[2,3] which seems to be a very appropriate tool in breeding programs, where many genetic lines need to be tested quickly and economically.

The objective of this work was to evaluate NIR spectroscopy as a tool to discriminate between whole intact seeds of yellow and narrow-leafed lupins. Additionally, the possibility of recognising several crosses or cultivars within each species was tested.

Materials and methods

A total of 1944 samples of intact seeds obtained from F2 plants belonging to six crosses of *L. angustifolius* ($n = 1764$) and from 15 cultivars of *L. luteus* ($n = 180$) were scanned in the Vis-NIR region with a NIRSystems 6500 monochromator (Silver Springs, MD, USA). Seeds were placed in ring cells, which were inserted in a spinning module for the readings. The software WinISI II (Infrasoft International, ISI, Port Matilda, PA, USA) was used for the scanning (and also for discriminant calibrations). Separate files were stored with the spectral data for each species and also for each cultivar or cross.

Separate discriminant models for species and genetic lines were developed by multivariate analysis (PLS2) by regressing spectral data to arbitrary values assigned by the model to each class (species, cultivar or cross) for the respective calibration. Different math treatments of the spectra were tested (derivative order, subtraction interval, smooth segment), with or without standard normal variate (SNV) and detrend.[4]

Cross-validation was used to select the best discriminant equation and to limit the number of terms.

Results and discussion

Species

The discriminant model was able to identify all samples of yellow and narrow-leafed lupin, without misses or uncertain results, although a few samples of yellow lupin (*L. luteus*) were recognised with a lower degree of certainty (Figure 1).

Samples predicted with a value equal to, or over 2, represent a perfect match, whereas values approaching 1.5 are uncertain and can go either way. This is consistent with differences in terms of chemical fractions found for these lupin species.[5]

Cultivars or lines

Results in Table 1 show that only one out of the 15 cultivars of yellow lupin evaluated was perfectly identified by the discriminant equation, i.e. obtained values over 1.5 for the correct class and less for other classes.

In five lines, over 80% of samples were correctly classified and in six lines there were no misclassifications. On the other hand, in four lines not a single sample was correctly classified.

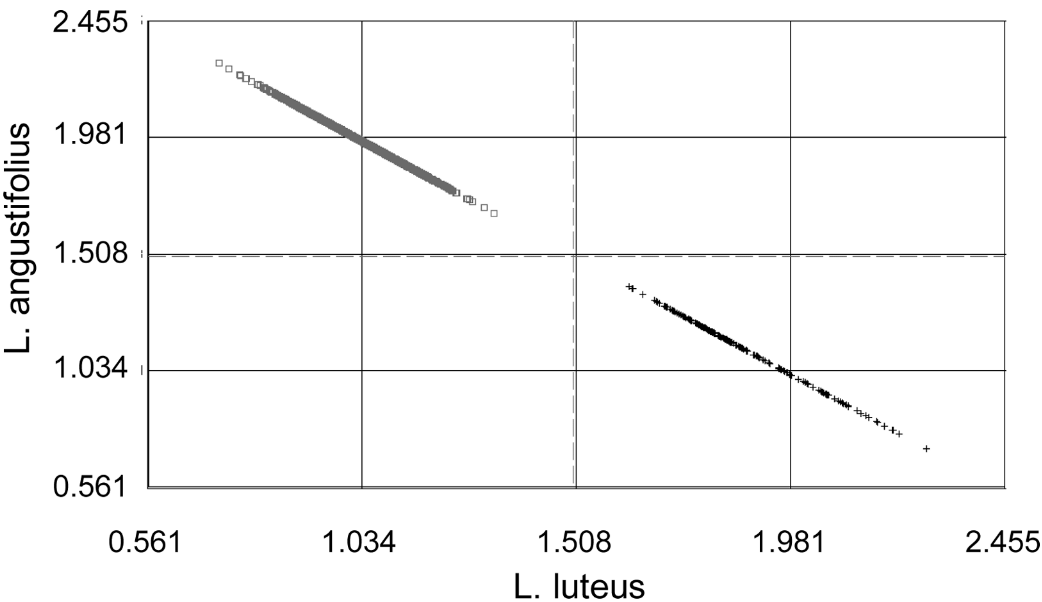

Figure 1. Scatterplot of predicted values in discriminant model, for *L. luteus* (x) and *L. angustifolius* (□).

Table 1. Percentage of samples correctly (hits) or incorrectly (failed) classified by discriminant equations developed for cultivars or crosses of yellow (*L. luteus*) and narrow-leafed (*L. angustifolius*) lupin.

Cultivars *L. luteus*	Hits (%)	Failed (%)	Cultivars *L. angustifolius*	Hits (%)	Failed (%)
1	16.7	56.7	1	66.4	15.5
2	25.0	25.0	2	96.0	0.7
3	0	75.0	3	84.0	4.0
4	83.3	0	4	74.7	8.0
5	83.3	8.0	5	94.0	0.7
6	58.3	8.0	6	86.8	3.3
7	83.3	0			
8	66.7	8.0			
9	0	42.0			
10	33.3	0			
11	0	66.7			
12	100	0			
13	0	25.0			
14	91.7	0			
15	75.0	0			

In the six crosses of narrow-leafed lupin, each comprising 110 samples to 151 samples, 66% to 96% of the samples were correctly classified, with different number of misses (1 to 17) i.e. a score below 1.5 for the correct class and over 1.5 for any other class; and also a range of uncertain samples (5 to 26). Calibration statistics are presented in Table 1.

Conclusions

NIR spectral data can recover signals characteristic of each species which, with proper discriminant models, can recognise seeds belonging to yellow or narrow-leafed lupin accurately. Although different cultivars or crosses within each species show spectral traits that in some cases allow correct classification, the certainty and accuracy is severely compromised.

References

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