Towards a zero toxin vetch *via* NIRS

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Introduction

Common vetch (*Vicia sativa* L.) is a remarkably adaptable, extremely diverse, self-pollinated legume, widely grown in both Northern and Southern hemispheres. In Australia, it provides a useful high protein (~28% c.p) grain for livestock. Nevertheless, it has long been known (Tschiersch 1963,[1] Bertrand, 1906,[2] Ressler *et al*. 1969,[3] Ressler and Tatake 2001[4]) to contain anti-nutritional factors (ANFs), which include cyanogenic glycosides, the favism toxins vicine and convicine, and of particular interest in the current study, the known neurotoxins beta-Cyano-L-Alanine and its gamma-L-Glutamyl dipeptide. The dipeptide is the major toxin (>95%), but the MIR nitrile absorbances are similar. A white seeded selection was obtained by the late Dr. Doza Chowdhury by crossing a farmer's toxic, white seeded, natural mutant of the well adapted cultivar known as Languedoc, with an Iranian low toxin line (IR28). The white seeded selection ("LOVE 2") from this cross reduced the toxin compared to the parental Jericho White by approximately 50%. Progress to date in lowering the toxin by a further 50% in a cross of Love 2 with a Bulgarian line (WV 24/1) to produce low toxin selections for a dark seeded Love 3 has relied upon recurrent selections up to F7, of the first two seeds in a single pod. The need for a non-destructive, single seed NIR analysis to progress towards the zero-toxin, common vetch has led to the current study.

Materials and methods

A synthetic standard curve over the range 0–3.2% w/w of the crystalline ammonium salt of gamma-L-Glutamyl-beta-Cyano-L-Alanine (Toxin) was adsorbed on cyclone-milled, denatured (120C/20min), red split lentils. The MIR data was collected with a Perkin Elmer Spectrum One FT-IR spectrometer. A cross-validated, partial least squares regression (PLSR) for 1 component, was fitted to the nitrile absorbance peak (2300–2200 cm^{-1}) using GRAMS AI /PLSplus (7.02). White (n=11) and dark (n=16) seeded vetch lines were tested for Toxin content using this method. The minimum toxin in both white and dark seeded lines was 0.5%. Toxin levels were further lowered by autoclaving a subset of white ($n=4$) and dark ($n=3$) seeded lines. Autoclaving was conducted at 121°C for sufficient time to reduce the Toxin % to zero. Samples were scanned on a FOSS XDS Near-Infrared Rapid Content Analyser as whole grain in the Round Cup. The reflectance spectra were collected over the range 400–2500nm at 2nm interval, 16 sample scans. To

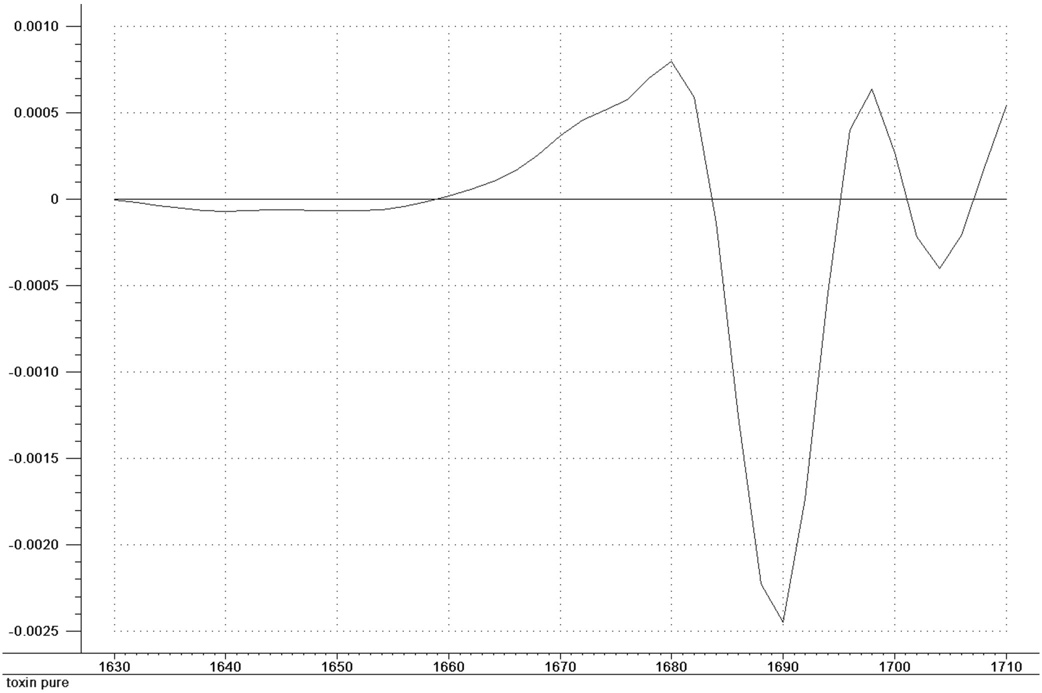

Figure 1. Spectrum of crystalline ammonium salt toxin.

simulate a spinning cup, samples were rotated 90° to obtain 4 sub scans and the averaged spectra were analysed using The Unscrambler v9.8 software. The second derivatives of the spectra were obtained by Savitzky Golay method, smoothing points 3 and polynomial order 2. The crystalline ammonium salt of the toxin was also scanned and treated in the same manner.

Results and discussion

Correlation charts[5] show that nitrile absorbs in the ranges 1640–1700 nm and 1850–1960 nm. Water has a strong absorption in the region 1850–1960 nm and so this wavelength range was not considered for this study. The spectrum of the Toxin (Figure 1) show strong, sharp absorbance peaks/troughs in the range 1640–1700 nm as expected. The Toxin spectra was compared to the spectra of the white (Figure 2) and dark (Figure 3) seeded vetch. The peaks/troughs are much broader in the vetch spectra and were shifted. This effect is commonly seen in spectra of agricultural products. Autoclaving the samples did not significantly alter the vetch lines and the spectra collected. A concentration effect in this region was observed with lower % Toxin having lower absorbance, with more toxic lines showing larger peaks in the nitrile absorbance region.

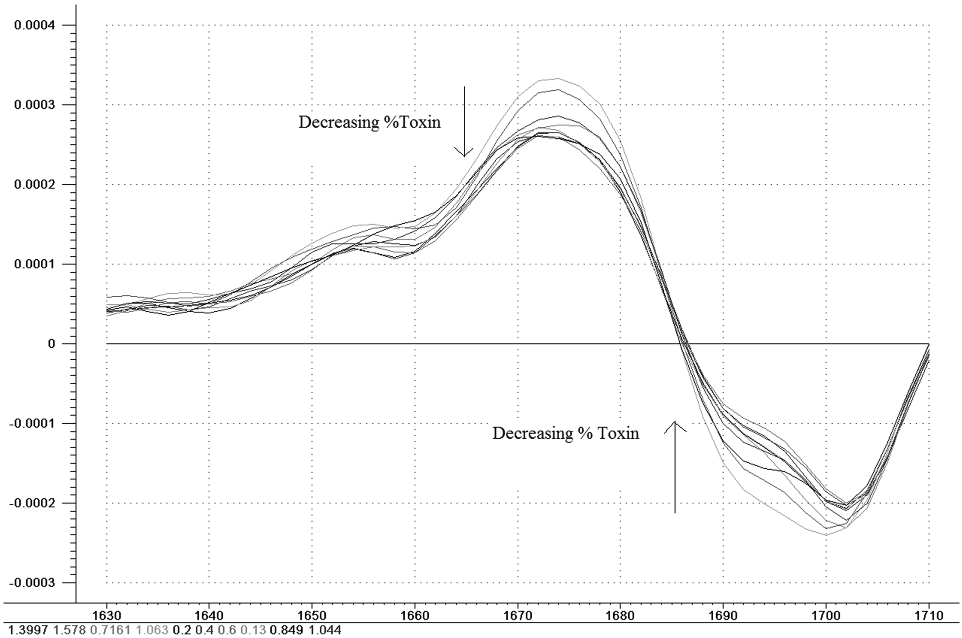

Figure 2. Spectra of white seeded vetch showing decreased % Toxin, decreased absorbance.

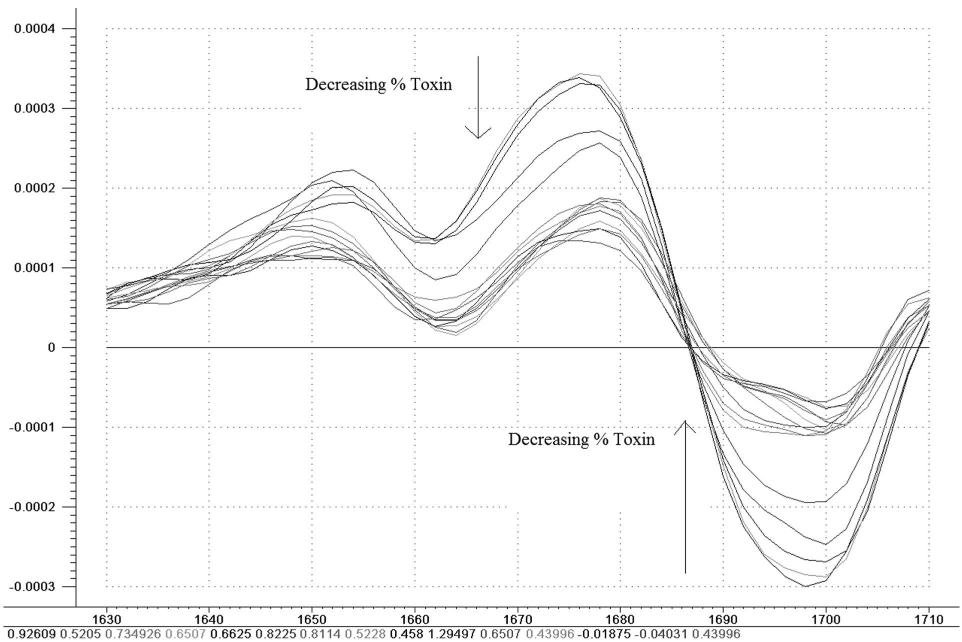

Figure 3. Spectra of dark seeded vetch showing decreased % Toxin, decreased absorbance.

Conclusion

This preliminary study shows promise and with more samples, a calibration model quantifying % Toxin in whole grain vetch should be achievable. The wavelength range for nitrile could be used and this would negate any interference from other components present in vetch. Individual calibrations would need to be developed for white and dark seeded lines as the spectral profiles are different. The concentration effect is present for both white and dark seeded vetch with decreasing % Toxin having decreasing absorbencies. The study is ongoing and further work is being conducted to develop bulk and single seed NIR % Toxin calibrations for common vetch.

References

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