Collaborative development of near infrared calibrations for quality testing of wheat and barley breeding material: 1. Optical matching of instruments

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Introduction

In July 1996, the Grain Industries Centre for NIR was established in Australia to carry out strategic research, to bring near infrared (NIR) researchers together into a network and to provide them with a support platform. Six of the Centre participants are quality testing laboratories associated with wheat and barley breeding programmes. NIR is already in routine use in these laboratories to replace some of the chemical tests (protein, moisture, hardness) required for quality assessment of breeding material. However, it has the potential to be used for the direct prediction of functional properties, i.e. for wheat: flour yield, damaged starch, water absorption, dough development time, extensibility and starch paste viscosity and for barley: β-glucan and malt extract. The availability of whole grain instruments provides a renewed incentive to develop the use of NIR to improve efficiency in quality screening of early generation lines where the numbers and quantity of samples preclude the use of traditional methods.

One of the difficulties in developing calibrations for plant breeding material is that by the stage when samples are available in sufficient quantities for laboratory testing some selection has already taken place. Thus, the ranges of properties of interest have been narrowed. The vision for two parallel projects on wheat and barley is to produce a wider range of material than would otherwise be available to any single breeding programme. This will be achieved by growing genetically diverse material, in-

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cluding lines that would otherwise have been discarded on quality grounds, over a wide range of environments in the collaborating breeding programmes. The philosophy was to optically match the instruments using the Shenk–Westerhaus single sample standardisation method[1] then record NIR data for each sample set in the laboratory of origin. These datasets will later be merged into a national spectral library for each commodity which will then be available for use by each collaborator.

Materials and methods

The instruments used were NIRSystems Model 6500 with sample transport mechanism. The standardisation was carried out using a set of seven wheat samples sealed in NIRSystems whole grain quarter cups. The samples were packed so that the surface remained unchanged during shipment. A further 34 samples of whole wheat with associated Kjeldahl protein data was used as an independent validation set.

The seven laboratories which participated in the standardisation are shown in Figure 1. The instrument located at North Ryde was designated as the Reference Instrument and the others as the Local Instruments. The sealed samples were scanned on the Reference Instrument before and after each Local Instrument in a star network. Care was taken to ensure that variations in temperature and humidity between laboratories were minimised.

Data were processed using Clone 1 within ISI NIRS3 v.4.1 software (Infrasoft International, Port Matilda, PA, USA). The median (middle one of the set of seven spectra when plotted on the log 1/*R* scale) spectrum of the seven sealed samples was used to create the standardisation file which was as-

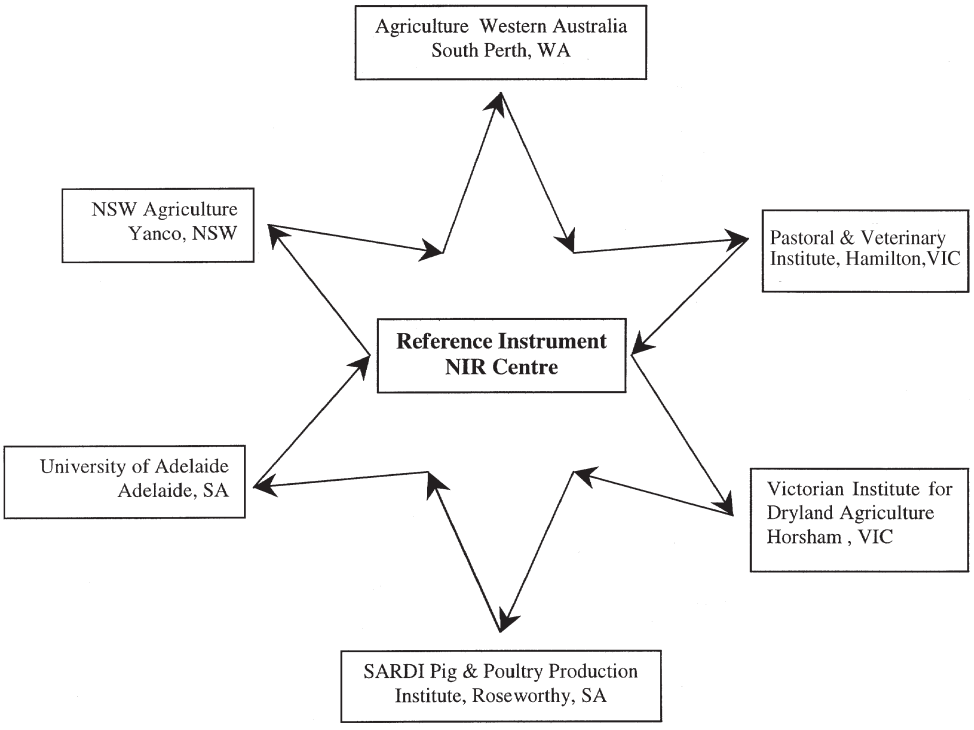

Figure 1. Laboratories and instruments in the Australian network.

Table 1. Results of whole wheat standardisation expressed as *RMS(C)*.

No.	1	2	3	4	5	6
2	194	—	—	—	—	—
3	144	186	—	—	—	—
4	213	303	237	—	—	—
5	243	163	273	344	—	—
6	216	179	183	350	222	—
7	440	476	466	393	481	480
Re-pack *RMS(C)* = 280 micro log 1/*R*						

sessed using the remaining six spectra.[1] The standardised spectra of all seven samples measured on the seven instruments were also merged to produce a repeatability file. The results of the standardisation were assessed in terms of comparison between the mean differences in spectra between instruments and between repeat measurements on the same instrument expressed as the average root mean square of differences of $d(\log 1/R)/d\lambda \times 10–6$ (8 nm gap, 4-point smoothing function), corrected for bias—*RMS(C)*. The criterion for a successful standardisation is for the average *RMS(C)* for the six test samples between each pair of instruments to be of the same order as that between re-packs on the same instrument.[2]

Results

The calculated *RMS(C)* values for each pair of instruments, following standardisation, are given in Table 1, together with the re-pack *RMS(C)* obtained from re-pack measurements on the 34 validation samples.

The standard error of prediction of protein content for the 34 validation samples, using a calibration derived on the Reference Instrument but applied to spectra measured on Local Instrument 3, was 0.28% which is close to the guideline *SECV* given in the Official Australian Method for protein in whole wheat by NIR.[3] The corresponding scatter plot is shown in Figure 2.

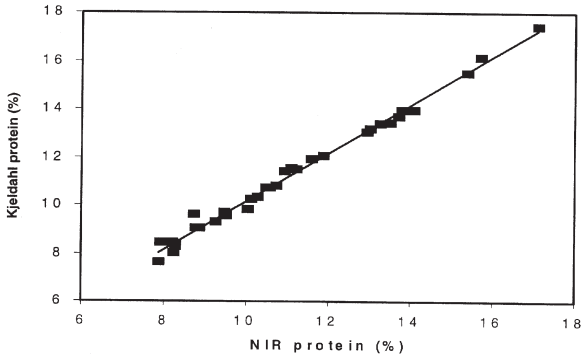

Figure 2. Plot of Kjeldahl versus NIR protein for 34 whole wheat samples.

Discussion

The procedure for creating a standardisation file using Clone 1 is mathematically and operationally very simple. The correction function is a series of offsets calculated from the difference in the log 1/*R* scale between the spectrum of a single sealed sample scanned on the Reference and Local Instrument. However, the conduct of the experiment requires careful attention to detail. The following points are especially important:

- The packing of the sealed samples is critical as the surface must remain constant during shipment and measurement.
- The diagnostics for each instrument in the network must be maintained within specifications.
- All instruments in the network must have the same type of sample cup and presentation system, for example coarse sample cell and transport mechanism.
- The temperature and humidity of each laboratory should not vary widely, although a standardisation file can be used to correct for this type of unmodelled effect.
- A star network (Figure 2) enables data to be referenced to the Reference Instrument between measurement on each Local Instrument. Furthermore, the replicate measurements obtained can be used to create the repeatability file.

According to the *RMS(C)* results (Table 1) and the performance of the protein calibration on the Local Instrument (Figure 2), the standardisation file was deemed successful in matching instruments. The result means to the Australian network that data from the different instruments can now be merged to produce calibrations based on a larger number of more diverse samples than would be available to any one laboratory, without the need for the actual samples to be moved around the country. In turn, the resulting spectral library and calibrations can be shared.

An additional benefit of standardisation is that, if any instrument requires a repair which results in a change to its characteristics, the standardisation file can be used to correct the spectra measured after the repair to match those measured before. In fact, Local Instrument 2 required a replacement lamp after the standardisation had been completed. The *RMS(C)* comparing the six whole wheat spectra before and after lamp replacement, without re-standardisation, was 376 micro log 1/*R*. After re-standardisation, the *RMS(C)* reduced to 155 for Local versus Reference Instrument and 168 for the Local before and after lamp replacement.

Historical data can also be shared provided it was generated as unstandardised ISI files using the same sample cell and assuming that the instrument performance has not changed significantly. It is possible to destandardise spectra when they have been modified with a previous std file which is not related to the new master. A procedure has also been devised to share data collected using NSAS (NIRSystems Inc., Silver Spring, MD, USA) software by treating the NSAS spectra as if they were generated on a different instrument and applying the Clone 1 standardisation. Although NSAS files can easily be imported into ISI, some additional manipulation was required to compensate for the different instrument setups required by the two programs.

Conclusion

At the time of standardisation, seven participants of the Grain Industries Centre for NIR had NIRSystems 6500 instruments with sample transport accessory and ISI software. These instruments have been standardised in order that spectra can be obtained when the same sample is measured on any of the instruments which are as similar as spectra obtained by re-packing the same sample on one instrument.

References

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2. P. Dardenne and R. Biston, in *Proc. 3rd International Conference on Near Infrared Spectroscopy*, Ed by R. Biston and N. Bartiaux-Thill. Agricultural Research Centre Publishing, Gembloux, Belgium, 655 (1991).
3. "Method No. 11.01: Determination of protein and moisture in whole grain wheat and barley by near infrared spectroscopy", in *Royal Australian Chemical Institute Cereal Chemistry Division Official Methods*. RACI, Melbourne (1998).