Office Use Only	
Project Code	
Project Type	

FINAL REPORT 2018

Applicants must read the *SAGIT Project Funding Guidelines 2017* prior to completing this form. These guidelines can be downloaded from www.sagit.com.au

Final reports must be emailed to admin@sagit.com.au as a Microsoft Word document in the format shown ***within 2 months*** after the completion of the Project Term.

PROJECT CODE	:	UA 316

PROJECT TITLE (10 words maximum)
New field-based tools to rapidly assess crop nitrogen and stress status

PROJECT DURATION

*These dates **must** be the same as those stated in the Funding Agreement*

Project Start date	1 July 2016					
Project End date	30 June 2018					
SAGIT Funding Request	2016/17		2017/18		2018/19	$

PROJECT SUPERVISOR CONTACT DETAILS

The project supervisor is the person responsible for the overall project

Title:	**First Name:**	**Surname:**	
Mr	Michael	Zerner	
Organisation:			
The University of Adelaide			
Mailing address:			
Telephone:	**Facsimile:**	**Mobile:**	**Email:**

ADMINISTRATION CONTACT DETAILS

The Administration Contact is the person responsible for all administrative matters relating to the project

Title:	**First Name:**	**Surname:**
Ms	Chelsea	DuBois

Organisation:
The University of Adelaide
Mailing address:

Telephone:	**Facsimile:**	**Mobile:**	**Email:**

PROJECT REPORT

Provide clear description of the following:

Executive Summary (200 words maximum)

A few paragraphs covering what was discovered, written in a manner that is easily understood and relevant to SA growers. A number of key dot points should be included which can be used in SAGIT communication programs

The purpose of this research project was to provide an initial investigation into the capability of field-based NIR (Near infra-red) to provide non-destructive, real-time results for plant N content and WSC (water soluble carbohydrates). Using the data obtained from the spectral reflectance measured in the field, N tissue content could successfully be predicted using the spectral reflectance information. The accuracy of this NIR based calibration was not accurate enough to directly be substituted for lab-based testing, but the result was suitable for large scale plot screening or whole paddock mapping of N concentration.

The approach using field based NIR devices despite not being as accurate as a lab-based test is much faster and can be completed in the field, in real-time. This is a significant advantage in that so many more readings can be taken across the entire paddock enabling it to be mapped for N concentration, rather than targeting a small number of lab-tests in specific zones in the paddock. This research has potential to improve the efficiency and effectiveness of N inputs for grain growers.

WSC could not be reliably and accurately predicted in the field using the same procedures to provide information on crop WSC reserves for grain-fill.

Project Objectives

A concise statement of the aims of the project in outcome terms should be provided.

This project aimed to, using new field-based whole NIR spectrum technology determine plant total water soluble carbohydrates (WSC) and nitrogen content (N %) of wheat and barley in the field. The project focused on establishing calibration curves to enable the use of new field-based NIR devices to predict WSC and N% rather than conventional lab-based testing procedures.

Successful calibrations of field-based whole NIR devices will enable rapid, in-season crop monitoring of WSC and N%. Given the importance of stored assimilates such as carbohydrates for influencing grain yield and quality, such measurements will give a valuable insight into the plants ability to buffer against environmental stresses in a given season. This would provide an important in-season crop health status for grain growers and a useful phenotyping tool for plant breeders.

Overall Performance

A concise statement indicating the extent to which the Project objectives were achieved, a list of personnel who participated in the Research Project including co-operators, and any difficulties encountered and the reasons for these difficulties.

All project objectives were achieved to their full extent. No significant difficulties were encountered during the project. The project was completed by myself at The University of Adelaide with the assistance of casual technical staff. APAL with the assistance of Sean Mason (Agronomy Solutions) provided all lab-based plant tissue analysis. Some delays were encountered with achieving water soluble carbohydrates results as this was a non-standard lab test. Prediction models were created by myself, in consultation with Kenton Porker (SARDI) and Daniel Cozzolino (CQ University).

Key Performance Indicators (KPI)

Please indicate whether KPI's were achieved. The KPI's ***must*** *be the same as those stated in the Application for Funding and a brief explanation provided as to how they were achieved or why they were not achieved.*

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
2016 - Collect spectral data and plant samples from field sites.	Y	
2016 - Complete lab-based analysis of plant samples and analysis with spectral data to establish calibration curves for WSC and N contents.	Y	
2017 - Prepare results of field and lab analysis and submit progress report to SAGIT.	Y	
2017 - Collect spectral data and plant samples from field sites.	Y	
2017 - Complete lab-based analysis of plant samples and analysis with spectral data to verify calibration curves for WSC and N contents.	Y	

2018 - Prepare results of field and lab analysis and submit progress report to SAGIT.	Y	
2018 - Prepare all research findings for completion of final report.	Y	

Technical Information (Not to exceed **three** pages)

Provide sufficient data and short clear statements of outcomes.

Over the past two years of the project a large amount of data has been obtained to develop calibrations for the NIR prediction of total N content and water soluble carbohydrates (WSC) in wheat and barley. Over 1500 plant samples were taken and analysed for N content and WSC in conjunction with non-destructive field-based NIR spectroscopy and lab-based NIR spectroscopy in order to create these predictive NIR models. A range of field trials were used containing wheat and barley across multiple growth stages, in contrasting environments and N management strategies. This ensured there was sufficient range in N content and WSC data to develop the most accurate and robust predictive models possible. Over the duration of the project this included trials sampled from Roseworthy, Mintaro and Loxton with various sowing times and amounts of applied N. Plots were also sampled from differing crop row spacings from 9-inch to 12-inch to investigate any associated impact of varying ground cover that may influence the field-based NIR readings. This was to ensure the predictive models using NIR were as robust as possible, so that one model would potentially be suitable to all end-users.

Data analysis and interpretations was a crucial part of this research project to link NIR spectral data to actual N% and WSC values. Spectral data was analysed using software, The Unscrambler X (CAMO). This model development software is extremely powerful and was used for partial least squares (PLS) analysis in creating NIR spectra predictive models.

Initial data analysis of a single year's data as presented in each of the 2017 and 2018 progress reports showed very promising results, where a single model was applicable to both wheat and barley in all tested environments. The next stage of the research was to analyse all data together to ensure the models hold true from one season to the next. Predictive NIR models were created for both N content and WSC using both field-based and lab-based, thus creating four models for comparison.

Firstly the calibration regression of dry, ground plant tissue samples (as prepared for wet chemistry) for total N content was very accurate when including all the data collected over the two year period. The R-value was 0.96 with a predictive error of 0.35% (Table 1 and Figure 1). This prediction accuracy is very good and provides a good option for fast, high through-put method for prepared samples. It also shows a single calibration model is applicable across wheat and barley. The residual predictive deviation (RPD), is defined as the ratio between the standard deviation of the population (SD) and the standard error in cross validation for the NIR predictions. The RPD was used to evaluate the predictive ability of the calibration models developed. The higher the value of the RPD, the greater the probability of the model to predict the chemical composition accurately in samples outside the calibration set. An RPD value greater than 5 (range 5 – 6.4) is considered good for quality control, while an RPD value between 3 and 5 is considered good for screening applications (Fearn 2002 and Williams 2001). The RPD for N content using ground samples was 4.4 (Table 1). This result is that this predictive calibration model is not of the standard required for quality control, but remains at a high level of accuracy for screening applications. The accuracy of the calibration regression for WSC using ground samples was significantly lower in accuracy than N content. This reduced accuracy was expected due the variable nature of WSC. The R-value was 0.66 with a predictive error of 56 g/kg and RPD equal to 1.26 (Table 1). This research was expected to produce a better outcome than this, as currently there is too much error in this predictive model to predict WSC. Figure 2 shows the large scatter in data points were predicted values are fitted against the reference points. It appears there may have been an error in the NIR scanning procedure, in that the correct NIR spectra is not aligned to the correct WSC lab result for all samples causing an inaccurate result. This is confirmed when only analysing one year of data as the R-value improves to 0.94 and the predictive error is halved to 24 g/kg (Table 2). The RPD from 2017 data alone was 3, hence the predictive model then becomes graded useful for screening purposes and provides a result that was more likely expected.

The use of the hand-held FieldSpec NIR device to provide non-destructive predictions of N content and WSC was the most important part of the project in relation to a greater industry significance as it presents as a new method of crop diagnostics for growers. Initial results including only one year of data provided some excellent early result. For example predictive model regression using 2017 data alone produced and R-value of 0.94 and predictive error of 0.5% (Table 2). This excellent result progressed well when all data was incorporated into the NIR predictive model, where from the regression an R-value of 0.9 was obtained with a predictive error of 0.64% and RPD equal to 2.41 (Table 1 and Figure 3). This result indicated the model is potentially suitable for screening applications of N content. This model includes wheat, barley, varied growth stages, contrasting canopy cover and sizes and all data is suitable to be included together. This is a very good result, as the robustness of the predictive model enables the one model to be used in all situations and environments rather than having a specific model for each scenario. The model can be improved for specific seasons (Table 2) or environments for example, but a single robust model applicable to all situations is preferred otherwise new calibrations would have to be constantly updated for specific conditions.

The current ability to predict N content using the whole spectra with such devices as the hand-held FieldSpec is a significant improved on the current NDVI sensors available. As instead of using only 2-3 specific wavelengths, the method used in this project uses every wavelength from 350-1100 nm. This provides much more information relating to the chemical composition of the crop canopy compared to just how green it is. Figure 5 shows the loadings relating to each wavelength included in the model. Peaks and troughs at approximately 690nm and 740nm highlight the region where NDVI is calculated, but there are also significant regions at 410, 920 and 950nm that are having a strong influence on the prediction of N content.

The WSC result did not have the same level of accuracy as N content. The R-value for WSC using the FieldSpec hand held device was 0.82 with a predictive error of 39 g/kg

(Table 1 and Figure 4). This increased error then resulted in a lower RPD of 1.78, which is below the screening threshold. This result would not enable any reliable use of this device to create plant stress related indices in its use as a measure of stored assimilates in the plant. Accuracy can be marginally increased if the data set becomes more specific to the particular environment or trial provided there is sufficient range in data values to create the calibration regression (eg, 2017 data in Table 2). The current regression may still have potential as a selection/screening tool for plant breeders, as it would be sufficient to categorise varieties or treatments into high or low ability to store assimilates for example.

No further research has been conducted to highlight the link between stored WSC and grain yield due to the current inability to accurately predict WSC using the hand-held NIR device. Following on the success of the NIR prediction of N content, preliminary investigations of the relationship to grain protein and the ability to predict it using NIR. A small barley trial where grain proteins had been obtained was used in a preliminary study to identify whether protein could be predicted prior to harvest using NIR. The predictive NIR model used the spectral data obtained from the trial plots up to anthesis and used this information to predict grain protein at maturity. The model was successful in predicting protein in this particular trial with a predictive error of 0.4%, as shown in Figure 6. This shows there is significant potential for NIR devices to be used in predicting grain protein prior to harvest. Further investigation is warranted and is likely to be difficult to create a single working model as environmental conditions can interact strongly during grain-fill causing variation in the concentration of grain protein making NIR predictions difficult.

(Please see attachment at the end of the report for Tables and Figures)

Conclusions Reached &/or Discoveries Made (Not to exceed one page)

Please provide concise statement of any conclusions reached &/or discoveries made.

The purpose of this research was to improve the ease of measurement of plant trait components, water soluble carbohydrates (WSC) and nitrogen content. The current standard method to measure WSC and N content relies on destructive plant tissue samples from the field and later dried and prepared for lab analysis. The use of Portable NIR (Near infra-red) were investigated to provide non-destructive, real-time measurements in the field. The use of a hand-held FieldSpec spectrometer was used to create field based calibrations for N content and WSC. A lab-based microNIR spectrometer was also used for comparison on the lab prepared samples.

Lab-based NIR provided an accurate result for the prediction of N content from prepared plant tissue samples. WSC was much more variable in the NIR predictive ability and further research would have to be undertaken to develop a more accurate NIR calibration model.

The N content calibration regression developed for the field-based NIR sensor was less accurate than the corresponding model developed in the lab. Despite this the calibration model could be used to estimate N from a screening level of accuracy with an error of 0.6%. This level of accuracy could be used to screen a large number of plots, or map the variation in N tissue concentration across a paddock. Although not equivalent to a lab diagnostic accuracy level the NIR predictions could be used to comfortably distinguish nutritional zones within the paddock for improved management of nitrogen fertilisers. The ability to have measurements conducted in field in a matter of seconds, enables many more measurements to be taken and provide much more information across the entire paddock rather than targeting a single test in specific zones as currently practiced with tissue testing.

WSC, as observed in the lab was much less accurate to predict using NIR in the field. The current NIR predictive ability using the developed model in this project is only capable of providing comparisons of high and low levels of WSC. The level of accuracy is not sufficient to be used as a diagnostic tool for measuring WSC.

Intellectual Property

Please provide concise statement of any intellectual property generated and potential for commercialisation.

The successful development of calibration regressions for non-destructive, in-field measurements of water soluble carbohydrates and N content using NIR may have the capacity to generate IP. With further research and development following this initial study, this IP has the potential to be commercialised through the development of a testing service that could be made publically available to growers and researchers.

Application / Communication of Results

A concise statement describing activities undertaken to communicate the results of the project to the grains industry. This should include:

- *Main findings of the project in a dot point form suitable for use in communications to farmers;*
- *A statement of potential industry impact*
- *Publications and extension articles delivered as part of the project; and,*
- *Suggested path to market for the results including barriers to adoption.*

Note that SAGIT may directly extend information from Final reports to growers. If applicable, attach a list of published material.

Key findings:

- **Successful calibration for predicting total N content from field and lab-based NIR devices in wheat and barley.**

- **Accurate and robust predictive NIR calibrations enable a single calibration to be applicable to both wheat and barley.**

- **Predictive NIR model for N content is consistent across seasons and contrasting environments.**

- **Water Soluble carbohydrate calibrations were successful but predictive accuracy was found to be low.**

Over the past year after promising initial results the project has been presented to vast range of audiences. Presentations were made at the SAGIT annual update, MSF field day at Loxton, National Frost Initiative annual meeting and at the Australian Barley Breeders meeting. A small set of results were also published and presented at the Australian Agronomy Conference held at Ballarat by Sean Mason, Agronomy Solutions. The project was also publicised in the Stock Journal, via the SAGIT column.

Findings of this project will represent a large technological advancement in crop phenotyping and diagnostics. Although not yet completed, it is planned to publish the findings of this project in a scientific journal as part of this project. Originally the project set out with a focus on building calibrations to predict WSC. Unfortunately these predictions were not as accurate as anticipated, but despite this the N% were found to be accurate and robust and may have a large impact on improving nitrogen management efficiencies.

Currently as part of the project, an accurate and robust calibration model has been created that has the potential to be used by growers to manage N inputs more accurately than using current N sensors. The current barrier is developing a suitable technology platform to make this technology accessible to growers. For example whether it's a NIR device with the predictive N models built-in to the device or a sensor with a cloud-based data platform which returns raw NIR data with N content outputs. Therefore a suggested path to market would potentially involving a NIR/precision agriculture based partner to assist in developing this work further to make it available to growers

POSSIBLE FUTURE WORK

Provide possible future directions for the research arising from the project including potential for further work and partnerships.

The success of the project has opened up many more research opportunities in the spectroscopy field relating to plant nutrition and health. From a nutrition perspective, NIR may be capable of predicting other plant macro and micronutrients to provide a more complete plant nutrition diagnostic tool. Therefore investigation into other nutrients could be undertaken in a similar manner as what has been done for nitrogen. This research project during its duration has collected substantial amounts of data additional to the project requirements. Given the small time allocation of the principal investigator, there still remains significant amounts of data yet to be investigated for this report. Plant tissue samples taken have been stored and could be used for further tissue analysis and use existing NIR data to identify the capabilities of its use for other nutrients.

Following on from plant tissue nitrogen content there was some relationship with grain protein, in that NIR showed signs of predictive capabilities. Again further investigation is warranted as accurate predictions of grain protein prior to harvest would be of great benefit to growers enabling to maximise grain prices through using on farm quality segregations.

Following on the research already conducted there is potential for partnerships to be forged into further development of this work in making it commercially available to growers in a user friendly format.

AUTHORISATION
Name: Michael Zerner
Position: Research Associate
Signature:
Date: 30/08/2018

Submit report via email to admin@sagit.com.au as a Microsoft Word document in the format shown ***within 2 months*** after the completion of the Project Term.

Supporting Attachment

Table 1. Summary of all data and model outputs (2016 & 2017) included in the total nitrogen and water soluble carbohydrates NIR calibration models (MicroNIR – lab NIR, ground samples and FieldSpec – field-based NIR).

		FieldSpec N% Data	FieldSpec WSC Data	MicroNIR N% Data	MicroNIR WSC Data
Predictive model summary	PLS Factors	12	14	4	3
	R	0.90	0.82	0.96	0.66
	R^2	0.82	0.68	0.94	0.44
	Prediction Error (±)	0.64	39.9	0.35	56.2
	RPD	2.41	1.78	4.40	1.26
Summary statistics of data included in each model	Mean	3.11	94.29	3.11	94.29
	Standard Deviation	1.54	71.01	1.54	71.01
	Minimum	0.80	0.0	0.80	0.0
	Maximum	7.20	364.16	7.20	364.16
	Count	1547	1493	1547	1493

Table 1. Summary of all data and model outputs (2016 & 2017) included in the total nitrogen and water soluble carbohydrates NIR calibration models (MicroNIR – lab NIR, ground samples and FieldSpec – field-based NIR).

		FieldSpec N% Data	FieldSpec WSC Data	MicroNIR N% Data	MicroNIR WSC Data
Predictive model summary	PLS Factors	11	12	5	5
	R	0.94	0.90	0.98	0.94
	R^2	0.89	0.82	0.96	0.89
	Prediction Error (±)	0.50	30.9	0.31	24.01
	RPD	3.00	2.33	4.84	3.00
Summary statistics of data included in each model	Mean	3.35	97.10	3.35	97.10
	Standard Deviation	1.50	72.0	1.50	72.0
	Minimum	1.00	3.18	1.00	3.18
	Maximum	7.20	364.16	7.20	364.16
	Count	865	837	865	837

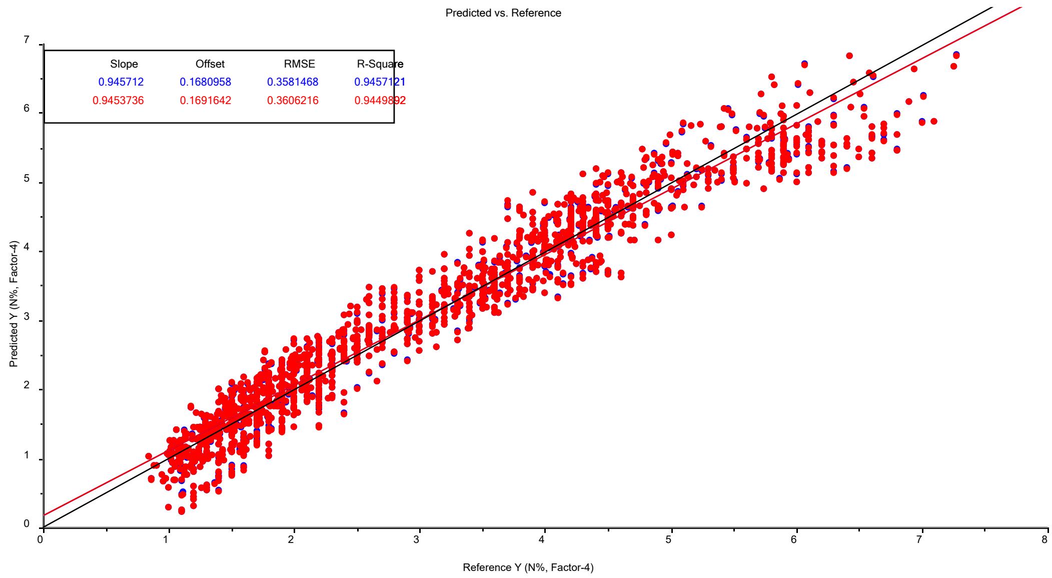

Figure 1. PLS regression calibration plots of NIR predicted total N content verses actual total N content using lab-based MicroNIR (900-1700nm) on dried, ground plant samples of wheat and barley at various growth stages across multiple locations during 2016 and 2017.

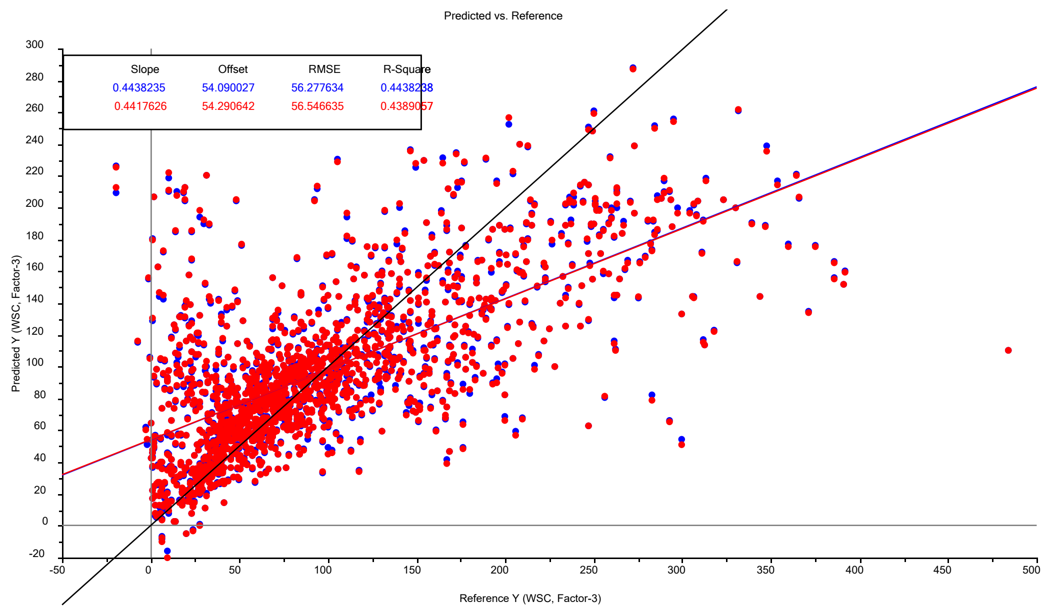

Figure 2. PLS regression calibration plots of NIR predicted WSC verses actual WSC using lab-based MicroNIR (900-1700nm) on dried, ground plant samples of wheat and barley at various growth stages across multiple locations during 2016 and 2017.

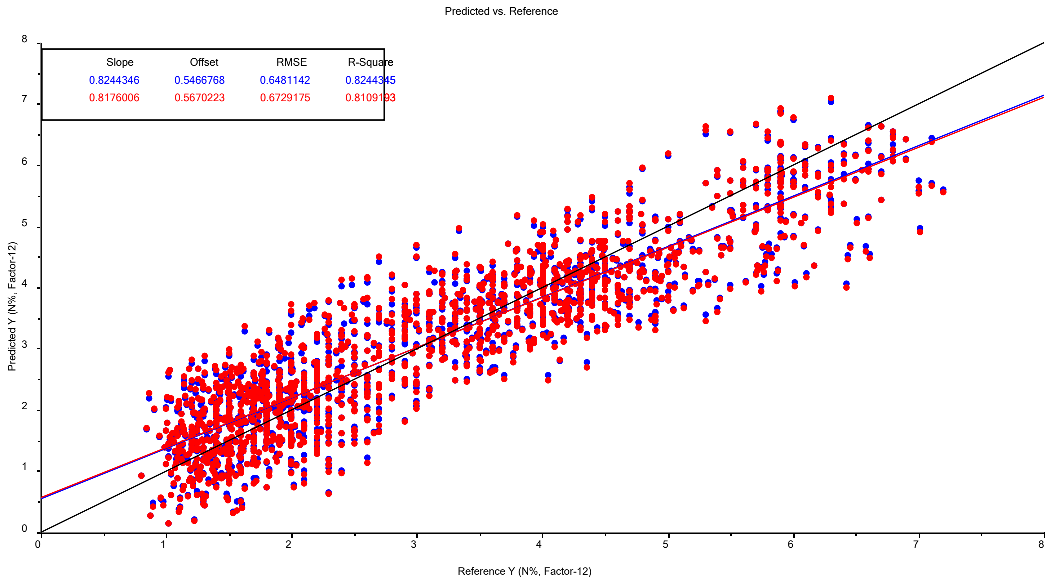

Figure 3. PLS regression calibration plots of NIR predicted total N content verses actual total N content using field-based FieldSpec (350-1100nm) on crop canopies of wheat and barley at various growth stages across multiple locations during 2016 and 2017.

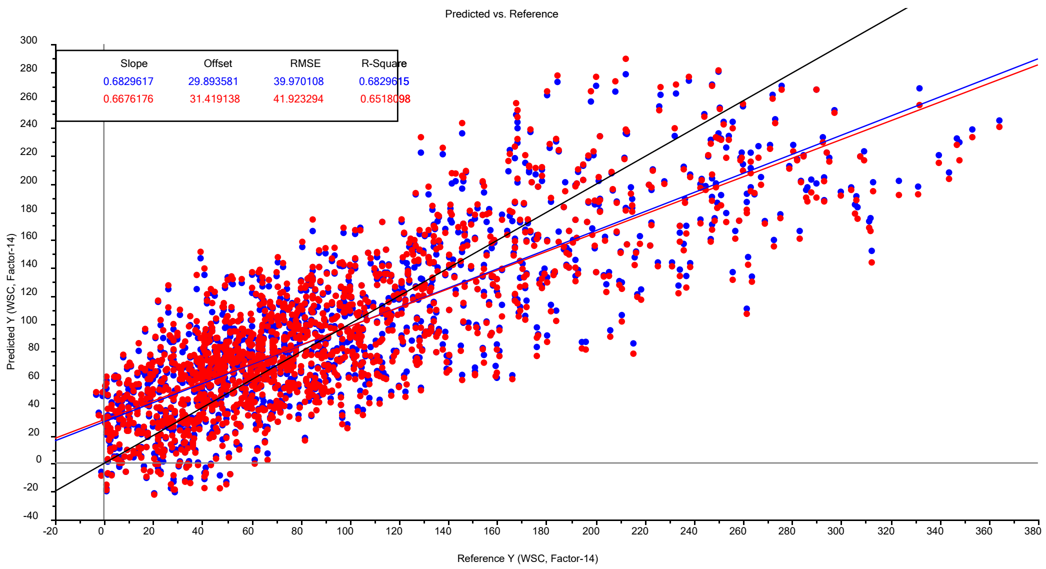

Figure 4. PLS regression calibration plots of NIR predicted WSC verses actual WSC using field-based FieldSpec (350-1100nm) on crop canopies of wheat and barley at various growth stages across multiple locations during 2016 and 2017.

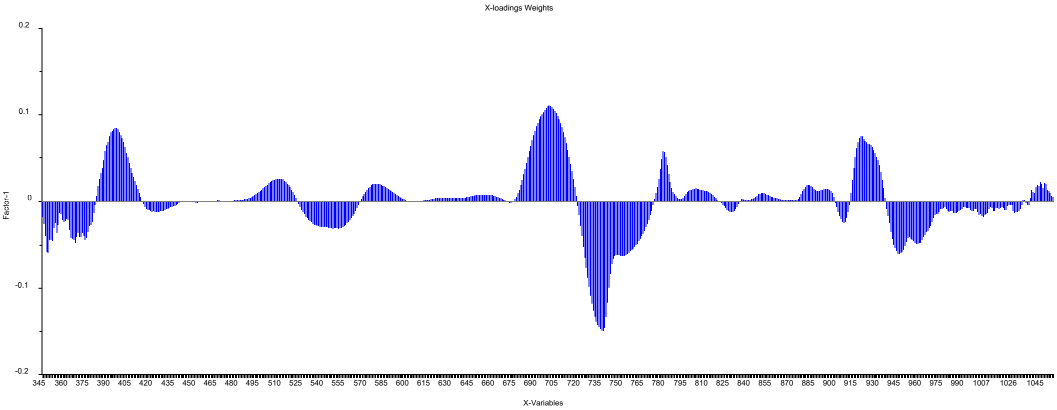

Figure 5. PLS regression calibration plots of NIR predicted total N content verses actual total N content using field-based FieldSpec (350-1100nm) on crop canopies of wheat and barley at various growth stages across multiple locations during 2016 and 2017.

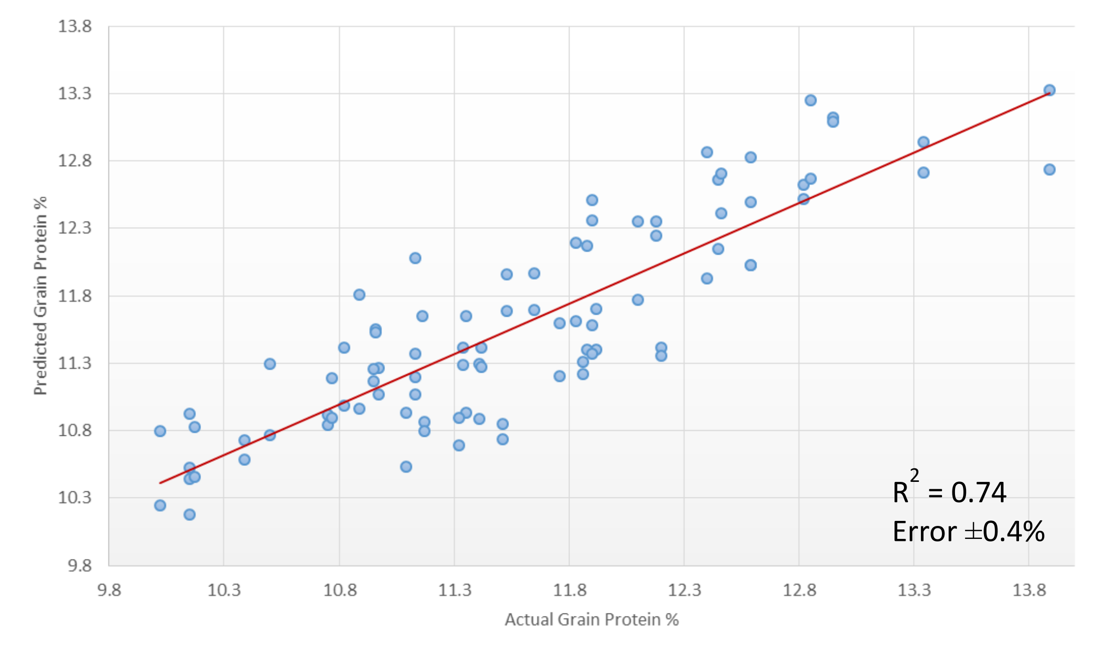

Figure 6. PLS regression calibration plots of NIR predicted grain protein verses actual grain protein using field-based FieldSpec (350-1100nm) on crop canopies barley at anthesis. Trial located at Roseworthy during 2016.