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Inversion of foliar biochemical parameters at various physiological stages and grain quality indicators of winter wheat with canopy reflectance

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Abstract. Canopy reflectance data selected at key growth stages of winter wheat were analysed. Regression equations between foliar total nitrogen content and other foliar biochemical concentrations, dried biomass indices, and grain quality indicators were established. The results showed that there were robust correlations between foliar total nitrogen content and other foliar biochemical concentrations, dried biomass indices, and grain quality indicators. The predictions of soluble sugar content, foliar water content, stem water content, foliar starch content, foliar dried weight, plant dried weight, and Leaf Area Index (LAI), etc. by foliar total nitrogen content were successful and feasible. The predictions of grain protein and dry gluten content by foliar total nitrogen content at anthesis were surprisingly good. The wavelength bands related to foliar total nitrogen content selected by regression equation were located at 1000–1140 nm and 1200–1300 nm.

1. Introduction

Inversion of biochemical concentration in plants by traditional field sampling methods is time-consuming and difficult to make for wide regional and global studies. It is highly desirable to develop new techniques to overcome the limitations of traditional field sampling methods. The application of remote sensing techniques to study and/or evaluate biochemical concentration in plants is a better way (Card *et al.* 1988).

Different studies and experiments demonstrated the usefulness and feasibility to estimate vegetation biochemical concentration using empirical regression equation models (Borel and Gerstl 1994, Fourty *et al.* 1994, Ganapol *et al.* 1998). A number of studies have also demonstrated relationships between foliar and canopy pigment content and ratios of narrow-band reflectance in the visible and 'red-edge' wavelengths (Thomas and Gausman 1977; Schutt *et al.* 1984). However, these studies have mostly focused on the chlorophylls (Yoder and Pettigrew-Crosby 1995, Blackburn 1998, Blackburn and Pitman 1999, Daughtry *et al.* 2000).

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Some approaches applied spectral reflectance measurements with different nitrogen treatments in corn and wheat (Blackmer *et al.* 1996).

There had been a few research works related to protein, sugar, starch, etc. Jacquemoud *et al.* (1995) used stepwise multiple regression analyses to estimate protein, cellulose, lignin and starch. Kokaly and Clark (1999) used band depth of absorption features at selected wavelengths to estimate foliar nitrogen, lignin and cellulose. Aber and Federer (1989) found that lignin concentration in green leaves was inversely related to annual nitrogen mineralization. However, there have been few studies concerning grain quality indicators forecasting by remote sensing.

Farmers, agricultural managers and grain processing enterprises are interested in measuring and assessing soil and crop status in order to apply adequate fertilizer quantities to crop growth, and thereafter, for health monitoring, and yield and grain quality forecasting during an advanced development stage of crop. For this purpose, hyperspectral remote sensing can play a vital role in providing time-specific and time-critical information for precision farming, due to its ability to measure foliar nitrogen variability.

2. Materials and methods

2.1. *Experimental site*

The experiment was conducted at the remote sensing base of Beijing suburb, which is located in Shunyi district (40°21′N, 116°34′E), for the year 2000–2001. The climate of the study is the north temperate zone with a mean annual rainfall of 507.7 mm and a mean temperature of 13°C.

2.1.1. *Soils*

Wheat was planted on a silt clay loam soil. The nutrients at 0–30 cm soil depth were as follows: organic matter 1.42–1.48%, total nitrogen 0.081–0.100%, alkali-hydrolysis nitrogen 58.6–68.0 mg kg^{-1}, available phosphorus 20.1–55.4 mg kg^{-1}, and rapidly available potassium 117.6–129.1 mg kg^{-1}.

2.1.2. *Variety*

The tested variety was '8138', a famous variety in China.

2.2. *Measured item and methods*

All canopy spectral measurements were taken from a height of 50 cm above canopy, under clear blue sky between 10:00 and 14:00 in Beijing Local Time, using an ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO, USA) fitted with 25° field of view fibre optics, which function in the 350–2500 nm spectral region with a spectral resolution of 3 nm at 700 nm and 10 nm in the 1400–2500 nm range, and with a sampling interval of 1.4 nm between 350 and 1050 nm, and 2 nm between 1050 and 2500 nm. Measurements over a 40 cm × 40 cm $BaSO_4$ calibration panel were used for calculation of reflectance. Vegetation and panel radiance measurements were taken by averaging 20 scans at optimized integration time with due care for dark current correction at every spectral measurement. At the same time, various field and laboratory data were collected for biochemical and geochemical analysis, such as chlorophyll, total nitrogen, relative water content, starch, soluble sugar and Leaf Area Index (LAI), etc.

Table 1. Correlation coefficients between foliar nitrogen content and spectral reflectance.

	Wavelength range (nm)						
	350–670	671–819	1000–1140	1200–1300	1301–1700	1700–2100	2100–2500
Correlation coefficient (r)	−0.05	−0.03	0.8325**	0.5138*	−0.4518	−0.3215	−0.2047

$r_{(0.05,\ 17)} = 0.456$; $r_{(0.01,\ 17)} = 0.575$.
*Significant at 5% level; **significant at 1% level.

3. Results

3.1. *Relationship between foliar nitrogen and spectral reflectance*

An empirical relationship between the chlorophyll content and total nitrogen content of leaves and canopy has been demonstrated (Wood *et al.* 1993, Yoder and Pettigrew-Crosby 1995, Pinar and Curran 1996). Many researchers have focused on the relationship between vegetation spectrum and chlorophyll content (Blackburn 1998, Blackburn and Pitman 1999, Daughtry *et al.* 2000). However, Chang *et al.* (1997) reported that there was no relationship between spectral reflectance and chlorophyll content. The total nitrogen content is often described by dried biomass, which is not affected by water in biology. In this study, we used foliar nitrogen content to indicate wheat nutrient status.

As shown in table 1, the correlation coefficients between spectral reflectance and total nitrogen were negative in visible and short-wave infrared, but positive in near-infrared in leaves. The bands in 1000–1140 nm and 1200–1300 nm were found to correlate with foliar nitrogen, as 1000–1140 nm band has a correlation coefficient of 0.8325, regression analysis of spectral reflectance in 1000–1140 nm and foliar nitrogen content carried out at different phenological stages of wheat. The results are presented in table 2.

Through the analyses presented in table 2, it is clear that there was good correlation between foliar nitrogen content and the logarithmic spectral reflectance at reviving and filling stage, the correlation between foliar nitrogen content and the reciprocal of reflectance spectrum at jointing stage was satisfactory. At ripening

Table 2. Regression equations between foliar nitrogen content and spectral reflectance (in the 1000–1140 nm region) at different growth stages.

Growth stage	Independent variable (x)	Dependent variable	Regression equation	Coefficient of determination (R^2)
Reviving stage	foliar nitrogen content (%)	ρ	$\rho = 5.8412x + 1.9519$	0.9132
		$1/\rho$	$1/\rho = -0.0049x + 0.0566$	0.7814
		$\ln\rho$	$\ln\rho = 0.1688x + 2.5958$	0.9657
Jointing stage	foliar nitrogen content (%)	ρ	$\rho = -1.6081x + 51.022$	0.6154
		$1/\rho$	$1/\rho = 0.0011x + 0.0181$	0.6879
		$\ln\rho$	$\ln\rho = -0.0415x + 3.966$	0.6647
Filling stage	foliar nitrogen content (%)	ρ	$\rho = -3.9018x + 54.915$	0.8012
		$1/\rho$	$1/\rho = 0.0038x + 0.0091$	0.7568
		$\ln\rho$	$\ln\rho = -0.1218x + 4.1777$	0.9325
Ripening stage	foliar nitrogen content (%)	ρ	$\rho = 6.1315x + 19.793$	0.9789
		$1/\rho$	$1/\rho = -0.0057x + 0.0427$	0.9127
		$\ln\rho$	$\ln\rho = 0.1877x + 3.0916$	0.9345

stage, the correlation between foliar nitrogen content and spectral reflectance itself was highly significant, since we should establish different regression equations through different forms of spectral reflectance to estimate the other indicators at different growth stages of wheat.

3.2. *Measured biochemical concentration and biomass*

The spectral-based inversion of plant biochemical concentrations has been studied by many researchers (Jacquemoud *et al*. 1995, Johnson and Billow 1996, Kokaly and Clark 1999). They only compared the correlation among biochemical concentrations. However, there exist different trends of biochemical concentrations at dynamic growth stages of wheat. The nutrition trend of different growth stages was discussed separately in this paper. The relationship between the biochemical concentration and spectral reflectance was analysed, and the regression equations were established. The results were as follows.

3.2.1. *Measured biochemical concentration and biomass at the reviving stage*

Fertilizing and watering at reviving stage significantly affect the canopy density. The correlation coefficients among biochemical concentration and dried biomass indices at reviving stage are listed in table 3. Here, the correlation between foliar nitrogen content and soluble sugar, leaf dried weight and LAI was significant, but inferior compared with other indicators. The regression equations based on the relationship between foliar nitrogen content and the foliar soluble sugar, foliar dried weight, and LAI are listed in table 4. The coefficients of determination in all the regression equations were very high. If the foliar nitrogen content is measured, the status of foliar soluble sugar, leaf dried weight, and LAI can be estimated by these regression equations.

3.2.2. *Measured biochemical concentration and biomass at the jointing stage*

Fertilizing and watering at the jointing stage significantly affect the grain yield. The correlation coefficients among biochemical concentration and dried biomass parameters at jointing stage are listed in table 5. It indicated that the correlation

Table 3. Correlation coefficients among biochemical concentrations and dried biomasses at the reviving stage.

	LTN	LCHL	LSS	LS	LWC	LAI	LDW	SDW	PDW
LTN	1								
LCHL	0.156	1							
LSS	−0.823**	−0.134	1						
LS	0.196	−0.204	−0.244	1					
LWC	−0.184	−0.331*	0.087	0.273	1				
LAI	−0.301*	0.350*	0.195	0.188	0.099	1			
LDW	−0.508**	0.152	0.369**	0.095	0.261	0.936**	1		
SDW	−0.012	−0.213	−0.323*	0.496**	0.512**	0.533**	0.533**	1	
PDW	−0.283	−0.045	0.006	0.349*	0.448**	0.827**	0.862**	0.888**	1

LTN, foliar total nitrogen; LCHL, foliar chlorophyll content; LSS, foliar soluble sugar content; LS, foliar starch; LWC, foliar water content; LAI, leaf area index; LDW, dry weight of foliar; SDW, dry weight of stem and sheath; PDW, dry weight of plant.

$r_{(0.05,\ 95)}=0.300$; $r_{(0.01,\ 95)}=0.351$.

*Significant at 5% level; **significant at 1% level.

Table 4. Regression equations between foliar nitrogen content and other indicators at the reviving stage.

Independent variable (x)	Dependent variable (y)	Regression equation	Coefficient of determination (R^2)
Foliar nitrogen content (%)	foliar soluble sugar (%)	$y=-6.8573x+50.068$ ($3.0\leqslant x\leqslant 7.0$)	0.7864
Foliar nitrogen content (%)	foliar dried weight (g)	$y=3.6329x-7.6274$ ($3.0\leqslant x\leqslant 7.0$)	0.7584
Foliar nitrogen content (%)	LAI	$y=0.0116x+0.6714$ ($3.0\leqslant x\leqslant 7.0$)	0.8864

between foliar nitrogen and soluble sugar, foliar water, and stem water content was extremely significant, but inferior compared with other indicators.

Regression equations based on the relationship between foliar nitrogen and soluble sugar, foliar water, and stem and sheath water content are listed in table 6. The coefficient of determination exceeded 0.80, and is 0.95 for the regression equation between foliar total nitrogen content and foliar water content. So it is feasible to estimate foliar water content, stem and sheath water content, and foliar soluble sugar content using foliar total nitrogen content at the jointing stage.

Table 5. Correlation coefficients among biochemical concentrations and dried biomass parameters at the jointing stage.

	LTN	LCHL	LSS	LS	LWC	LAI	LDW	SDW	PDW
LTN	1								
LCHL	0.238	1							
LSS	0.365**	0.022	1						
LS	0.026	0.288	−0.212	1					
LWC	0.418**	−0.199	−0.434**	0.139	1				
LAI	−0.017	0.186	−0.420**	0.233	0.397**	1			
LDW	−0.13	−0.064	−0.189	0.266	0.103	0.259	1		
SDW	−0.096	−0.287	0.012	0.008	0.187	0.29	0.827**	1	
PDW	−0.121	−0.165	−0.109	0.165	0.144	0.284	0.969**	0.940**	1

$r_{(0.05,\ 95)}=0.300$; $r_{(0.01,\ 95)}=0.351$.
*Significant at 5% level; **significant at 1% level.
For abbreviations, see table 3 footnote.

Table 6. Regression equations between foliar nitrogen content and other indicators at the jointing stage.

Independent variable (x)	Dependent variable (y)	Regression equation	Coefficient of determination (R^2)
Foliar nitrogen content (%)	foliar soluble sugar (%)	$y=2.789x-9.379$ ($4.0\leqslant x\leqslant 8.0$)	0.8025
Foliar nitrogen content (%)	foliar water content (%)	$y=1.7691x+66.98$ ($4.0\leqslant x\leqslant 8.0$)	0.9562
Foliar nitrogen content (%)	stem and sheath water content (%)	$y=2.0786x+68.408$ ($4.0\leqslant x\leqslant 8.0$)	0.8421

Table 7. Correlation coefficients among biochemical concentrations and dried biomasses at the filling stage.

	LTN	LCHL	LSS	LS	LWC	LAI	LDW	SDW	PDW
LTN	1								
LCHL	0.296	1							
LSS	0.704**	0.648**	1						
LS	0.086	0.09	−0.169	1					
LWC	0.393**	0.502**	0.415**	0.075	1				
LAI	0.222	−0.263	−0.144	−0.17	0.066	1			
LDW	0.367**	−0.078	0.023	−0.115	0.055	0.947**	1		
SDW	0.314*	0.083	0.169	−0.287	0.05	0.563**	0.674**	1	
PDW	0.351**	0.044	0.139	−0.258	0.055	0.710**	0.814**	0.978**	1

$r_{(0.05,\ 95)} = 0.300$; $r_{(0.01,\ 95)} = 0.351$.
*Significant at 5% level; **significant at 1% level.
For abbreviations, see table 3 footnote.

3.2.3. *Measured biochemical concentration and biomass at the filling stage*

Some researchers reported that fertilizing and watering at the filling stage significantly affected the grain protein content and enhanced the grain quality (Chang and Jiang 1996, Jing *et al.* 1999). Table 7 lists the correlation coefficients of each biochemical concentration and dried biomass indicators at the filling stage. As shown in table 7, the correlation coefficients between foliar nitrogen content and soluble sugar, stem and sheath water leaf dried weight, stem and sheath dried weight and plant dried weight were extremely significant.

Regression equations between foliar nitrogen content and other biochemical concentrations at the filling stage are listed in table 8. The determination coefficients of the regression equations are very high. So the estimation of stem and sheath water content, soluble sugar content and plant dried weight through foliar nitrogen content is feasible.

3.2.4. *Measured biochemical concentration and biomass at the ripening stage*

The correlation coefficient between measured biochemical concentration and dried biomass index and the regression equations between foliar nitrogen content

Table 8. Regression equations between foliar nitrogen content and selected biochemical parameters at the filling stage.

Independent variable (x)	Dependent variable (y)	Regression equation	Coefficient of determination (R^2)
Foliar nitrogen content (%)	soluble sugar (%)	$y = -1.1723x + 9.8327$ ($4.5 \leqslant x \leqslant 6.0$)	0.7968
Foliar nitrogen content (%)	stem and sheath water content (%)	$y = -10.998x + 134.65$ ($4.50 \leqslant x \leqslant 6.0$)	0.8521
Foliar nitrogen	content (%)foliar dried weight (g)	$y = 3.3532x + 21.324$ ($4.50 \leqslant x \leqslant 6.0$)	0.7968
Foliar nitrogen content (%)	stem and sheath dried weight (g)	$y = 12.535x + 108.21$ ($4.50 \leqslant x \leqslant 6.0$)	0.8567
Foliar nitrogen content (%)	plant dried weight (g)	$y = 15.888x + 129.6$ ($4.50 \leqslant x \leqslant 6.0$)	0.9124

Table 9. Correlation coefficients among biochemical concentrations and dried biomasses at the ripening stage.

	LTN	LCHL	LSS	LS	LWC	LAI	LDW	SDW	PDW
LTN	1.000								
LCHL	0.592**	1.000							
LSS	0.126	0.535**	1.000						
LS	−0.345*	−0.308*	−0.314*	1.000					
LWC	0.576*	0.510**	0.616**	−0.500**	1.000				
LAI	0.197	−0.212	−0.383**	0.023	0.066	1.000			
LDW	0.305*	−0.207	−0.449**	−0.032*	0.055	0.947**	1.000		
SDW	0.274	−0.008	−0.391**	−0.362**	0.050	0.563**	0.674**	1.000	
PDW	0.302*	−0.065	−0.435**	−0.294	0.055	0.710**	0.814**	0.978**	1.000

$r_{(0.05,\ 95)}=0.300$; $r_{(0.01,\ 95)}=0.351$.
*Significant at 5% level; **significant at 1% level.
For abbreviations, see table 3 footnote.

and other biochemical correlations, at the ripening stage, are listed in tables 9 and 10, respectively. As shown in table 9, the correlations between foliar nitrogen content and chlorophyll content, foliar starch content, foliar water content, stem and sheath dry weight and plant dried weight are significant, but are lower for other biochemical concentration parameters.

From table 10, it can be seen that the coefficient of determination is high for the regression equations describing the relationship between foliar nitrogen and chlorophyll content, stem and sheath water content, foliar starch content, foliar water content, foliar dried weight, and plant dried weight, especially between foliar nitrogen and chlorophyll and stem and sheath water content. If the foliar nitrogen content is measured, the stem and sheath water content, foliar dried weight, stem and sheath dried weight and plant dried weight could be estimated by these regression equations; moreover, there is high reliability of the estimation of chlorophyll, stem and sheath, and sheath water content.

Table 10. Regression equations between foliar nitrogen content and selected biochemical parameters at the ripening stage.

Independent variable (x)	Dependent variable (y)	Regression equation	Coefficient of determination (R^2)
Foliar nitrogen content (%)	chlorophyll content (%)	$y=1.968x-2.6886$ $(1.0\leqslant x\leqslant 3.0)$	0.8120
Foliar nitrogen content (%)	foliar starch content (%)	$y=-6.0868x+27.4481$ $(1.0\leqslant x\leqslant 3.0)$	0.7687
Foliar nitrogen content (%)	foliar water content (%)	$y=4.4283x+57.371$ $(1.0\leqslant x\leqslant 3.0)$	0.7435
Foliar nitrogen content (%)	stem and sheath water content (%)	$y=5.204x+55.324$ $(1.0\leqslant x\leqslant 3.0)$	0.8432
Foliar nitrogen content (%)	foliar dried weight (g)	$y=5.9996x+14.868$ $(1.0\leqslant x\leqslant 3.0)$	0.7543
Foliar nitrogen content (%)	plant dried weight (g)	$y=19.511x+98.616$ $(1.0\leqslant x\leqslant 3.0)$	0.7612

Table 11. Correlation coefficients between foliar nitrogen content and the grain quality indicators.

Grain biochemical content	Protein content (%)	Flour dried gluten (%)	Sedimentation value (%)	Forming time (min)	Stable time (min)	Mixing tolerance (%)	Rupture time (min)
Foliar nitrogen content (%)	0.5265*	0.4940**	0.2343	−0.0114	−0.1540	−0.0351	−0.1253*

$r_{(0.05,\ 26)} = 0.374$; $r_{(0.01,\ 26)} = 0.478$.
*Significant at 5% level; **significant at 1% level.

3.3. *Foliar nitrogen content and grain quality indicators*

The grain quality of winter wheat are characterized by protein content, wet gluten and dry gluten content, sedimentation value, forming time, stable time and so on. The correlation coefficient between total nitrogen content at anthesis and grain quality indicators is high, as shown in table 11. Here, the correlation between foliar nitrogen content and protein, flour dried gluten were extremely significant.

The foliar nitrogen content at anthesis stage is used to establish the predictive regression equation to estimate grain quality. The prediction regression equation of protein content in grain through foliar nitrogen content at anthesis has the form ($n = 75$)

$$y = 8.1356x - 27.261\left(5 \leqslant x \leqslant 6,\ R^2 = 0.9012\right) \quad (1)$$

where x is the total nitrogen content in per cent in leaf at anthesis stage, and y is protein content in per cent in grain. The reliability of the regression equation can be seen in figure 1.

The prediction regression equation of flour dried gluten in grain through foliar nitrogen content at anthesis ($n = 75$) is

$$y = 6.8815x - 24.546\left(5 \leqslant x \leqslant 6,\ R^2 = 0.8579\right) \quad (2)$$

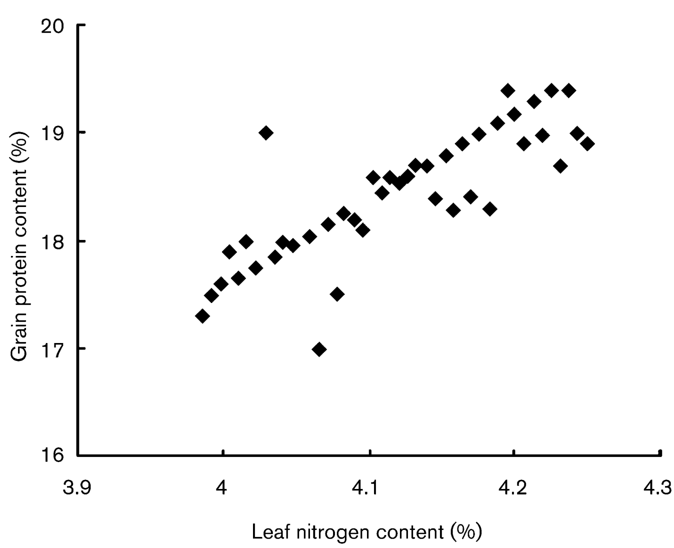

Figure 1. Relationship between foliar nitrogen content and grain protein content.

where x is total nitrogen content in the leaf at anthesis in per cent and y is dry gluten content in grain in per cent.

The grain protein content was estimated by regression analysis; the correlation between the simulated and the measured values can be seen in figure 2. The coefficient of determination between the simulated and the measured values is 0.9687, which is extremely significant. The results mentioned above indicate that the prediction of grain quality indicators by foliar nitrogen content is surprisingly good.

4. Discussion

4.1. *Relationship between wheat foliar nitrogen content and spectral reflectance*

Estimation of biochemical constituents in plants using the reflectance spectrum has been studied by many researchers (Stuedler *et al.* 1989, Aber and Federer 1992). Some researchers only compared the correlation among biochemical concentrations. However, there existed differences in correlation of biochemical concentrations and dried biomass at different growth stages. This made the biochemical concentration evaluation through remote sensing more difficult. Taking this situation into account, we examined a great number of samples, and all the established regression equations in this paper are based on these samples. The wavelength bands related to foliar nitrogen content are located at 1000–1140 nm and 1200–1300 nm, while it has very significant correlation in 1000–1140 nm.

4.2. *Relationship between foliar nitrogen content and grain quality indicators*

Remote sensing offers the potential to determine rapidly the physiological conditions of wheat in wide areas, and spectral reflectance measurement at leaf and canopy scales in the field provides a promising, expeditious and non-destructive way to gather information in a relatively wide area in a short time. With the development of space techniques, remote sensing is being broadly applied in agriculture. How to use remote sensing technology to predict wheat grain quality is an important problem. We have established a series of regression equations for predicting grain quality indicators (e.g. protein, dry gluten, wet gluten). The present

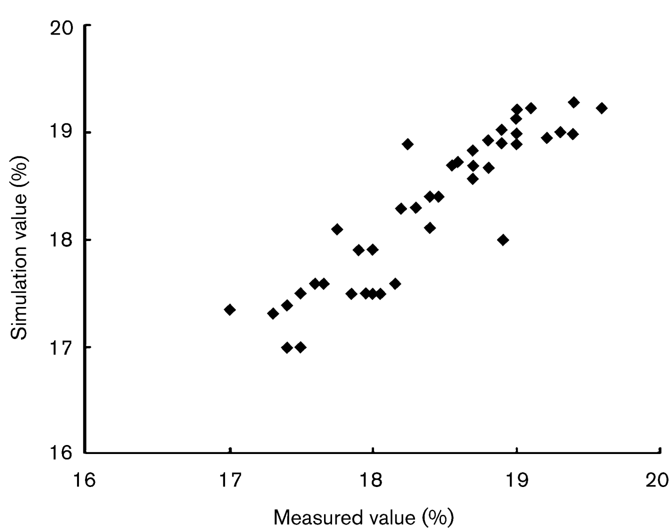

Figure 2. Relationship between simulated and measured values for grain protein content.

study has shown that there are robust correlations between foliar total nitrogen content and foliar biochemical concentrations and grain quality indicators. The predictions of grain protein, dry gluten, and wet gluten content by foliar total nitrogen content were surprisingly good.

5. Conclusion

The regression equations given in this paper have a strong potential for 'operational' use in the context of precision agriculture. They allow the on-site and non-sampling mode of crop-growth monitoring, fertilizing and water guiding, and grain-quality forecasting. Using these results, we are developing some simple instruments in these sensitive bands. For example, an optical camera lens focusing on 1000–1140 nm and 1200–1300 nm could be fixed on an agricultural machine travelling in the field. Such simple instruments can diagnose the plant growth status by the acquired spectral response. Moreover, a careful analysis should be carried out to investigate the effects of these characteristics: centre location, band width and sensor height above the ground.

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