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Research Article

Spectral Based Non-Invasive Estimation of Plant Chlorophyll Content

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ABSTRACT

Pot experiment was conducted with 10 wheat genotypes for understanding their behavior to different water stress levels. Hyperspectral data (350 to 2500 nm) and plant chlorophyll content were measured at different stress levels for identifying spectral indices and prediction models to develop non-invasive chlorophyll estimation protocols for a large number of genotypes. Evaluation of sensitive spectral indices was done with respect to leaf chlorophyll content and prediction models were developed. Models could satisfactorily predict the chlorophyll content of the samples, which were kept in same environment (R^2 ~ 0.29-0.75, 0.09-0.43 and 0.32-0.71, for chlorophyll a, b and total chlorophyll, respectively). The root mean square error between measured and predicted chlorophyll contents was the lowest for modified normalized difference index (mND_{705}). Among the normalized difference spectral indices (NDSI), NDSI_Chla (R_{700}, R_{762}), NDSI_Chlb (R_{660}, R_{764}), and NDSI_Total_Chl (R_{700}, R_{762}) satisfactorily predicted chlorophyll a, b and the total content, respectively. The retrieval of chlorophyll a and total content through our proposed indices was better than by using traditional vegetation indices, but in case of chlorophyll b, the indices were unsatisfactory in prediction. Newly developed and validated spectral algorithms specific to chlorophyll a and total chlorophyll content can further be used for nondestructive estimation of chlorophyll content in wheat.

Key words: Chlorophyll content, Relative water content, Water-deficit stress, Hyperspectral remote sensing

Introduction

The leaf chlorophyll is the most important plant pigment for photosynthesis (Blankenship, 1992), enabling conversion of light energy to chemical energy (Richardson *et al.*, 2002) and is directly related to plant stresses and senescence, (Gitelson and Merzlyak, 1994; Merzlyak *et al.*, 1999; Peñuelas and Filella, 1998) and leaf nitrogen (N) status (Cartelat *et al.*, 2005; Moran *et al.*, 2000; Ranjan *et al.*, 2012, Mahajan *et al.*, 2014). Estimation of chlorophyll content would lead to assess plant stress, nutritional state and relationships between plants and their environment, which is of great importance in agricultural field management (Zarco-Tejada *et al.*, 2004).

Conventional methods of chlorophyll estimation, through extraction and spectrophotometric or HPLC measurement are destructive, and are thus difficult for repated measurement of changes in pigments over time

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for a single leaf. In addition, the techniques are time consuming and expensive, thus making the overall assessment of vegetation health at large scale impractical (Sims and Gamon, 2002). In contrast, spectral reflectance based measurement is nondestructive, rapid, and can be applied across spatial scales (Gamon and Qiu, 1999). Spectral indices are considered as quantitative measurements indicating the vigor of vegetation (Bannari *et al.*, 1995). For decades, hyperspectral remote sensing applications described in the literature succeeded in deriving the relationships between leaf/canopy spectral reflectance and crop variables of interest (e.g., chlorophyll, water and N) through identifying spectral indices (Blackburn, 1998; Datt, 1998; Gitelson *et al.*, 2003, 2006; Peñuelas *et al.*, 1995; Sims and Gamon, 2002, Sahoo *et al.*, 2015). Transformed Chlorophyll Absorption Ratio Index (TCARI) or Optimized Soil-Adjusted Vegetation Index (OSAVI) integrates lower soil background effects and better sensitivity to chlorophyll concentration (Haboudane *et al.*, 2002). In this study, leaf chlorophyll indices were developed and tested using a large simulated database (11,583 spectra) of a leaf-radiative transfer model, PROSPECT along with ground truth to determine 'universal' chlorophyll content indices applicable to a wide range of species and leaf structures (Le Maire *et al.*, 2004). However, little information is available on the effect of water stress on chlorophyll content of wheat genotypes. The present study aims to develop spectral indices for prediction of the chlorophyll content of a large number of wheat genotypes exposed to different water stress levels through proximal diffuse reflectance measurement.

Materials and Methods

Study area

A pot experiment was conducted at the experimental farm, ICAR-India Agricultural Research Institute, New Delhi (28°38′23″ N and 77°09′27″ E; 228.6 m above mean sea level). The climate is classified as subtropical and semiarid, characterized by hot dry summer and cold winter. The mean daily minimum temperature during November to end of April (*rabi* season) ranged from 1 to 22°C while the mean daily maximum temperature from 12 to 41.2°C in 20013-14. A total rainfall of 163.6 mm only was received during *rabi* season.

The experimental (pot) soil is Typic Haplustept and sandy loam in texture, and has 0.41% organic carbon (Walkley and Black 1934), pH 8.3 and electrical conductivity of 0.47 dS m^{-1} in 1:2.5 soil to suspension ratio (Jackson 1973).

Experimental details

The experiment was conducted in *rabi* season, 2013 with 10 genotypes (stress sensitive and tolerant) of wheat each with 3 replications. During initial growth stage, watering was done every day, but withheld at flowering stage to impose the water-deficit stress. Spectral observations, relative water content (RWC) and chlorophyll content of leaves were measured on each day during the stress period till the plants dried completely.

Leaf spectral measurements

The reflectance of the flag leaves, randomly picked, were monitored at 350 to 2500 nm spectral range by using a field portable FS^3spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA) with an assembly of contact probe and clips. By pressing the contact probe on the leaf surface, the leaf surface was only illuminated by a constant light source inside the contact probe. Each leaf sample consisted of an overlapping pile of 2-3 leaves to eliminate the possible effect of the background on the spectra. A spectrally black surface was also put on the underside of the leaf to minimize the background reflectance effect. Prior to spectral reflectance measurement, the instrument was optimized with white reference panel (Spectralon of Labsphere, Inc., Sutton, NH, USA), and reference reflectance was measured followed by canopy reflectance measurements. Each spectral measurement was the average of 50 spectral scans of a sample.

Leaf chlorophyll content

After spectral measurements, leaves were precisely weighed to 50 mg and chlorophyll was extracted by a non-macerated method by equilibrating it with 10 ml DMSO (Dimethyl Sulphoxide) in a capped vial and keeping in an oven at 65ºC for 3 h (Hiscox and Israelstam, 1979). The decanted solution was used to estimate the absorbance at 645 and 663 nm wavelengths using Spectronic-20 Spectrophotometer. The leaf chlorophyll content was calculated by using the formula as given below (Arnon, 1949), and expressed as mg of Chl g^{-1} of leaf fresh weight).

$$\text{Total chlorophyll} = \frac{(20.2 \times A_{645} + 8.02 \times A_{663})}{(1000 \times W)} \times V \quad \ldots(1)$$

$$\text{Chlorophyll a} = \frac{(12.7 \times A_{663} - 2.69 \times A_{645})}{(1000 \times W)} \times V \quad \ldots(2)$$

$$\text{Chlorophyll b} = \frac{(22.9 \times A_{645} - 4.68 \times A_{663})}{(1000 \times W)} \times V \quad \ldots(3)$$

Here, V is the final volume (ml) of extract, W is the fresh weight of leaf sample and A_{645},A_{645} and A_{663} are the absorbance at 645 and 663 nm, respectively.

Measurement of relative water content

Once the fresh weight (FW) was obtained, the leaf sample was transferred to a petri dish, rehydrated in deionized water for 4 h until they became fully turgid at 25°C and the turgid weight (TW) was recorded. Finally the leaf sample was oven-dried at 60°C till constant weight was obtained, and the dry weight (DW) was recorded. The relative water content (RWC) was calculated using the following formula:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad \ldots(4)$$

Computation of spectral vegetation indices

Different hyperspectral vegetation indices (VIs) are presented in Table 1, and newly proposed indices (NDSI_Chla (R_{700}, R_{762}) and NDSI_Total_Chl (R_{700}, R_{762}) in the present study were evaluated for monitoring the chlorophyll content of plants. New indices were proposed as a normalized configuration of specific wavelengths showing the highest and the lowest significant correlations with the chlorophyll content. Simple regression models were calibrated-validated, and the coefficient of determination (R^2) and root mean square error (RMSE) of prediction were calculated to compare prediction accuracy of existing as well as newly developed vegetation indices.

Results and Discussion

Spectral reflectance of wheat crop at different water-deficit stress levels

Spectral signatures of leaves were distinct at different water-deficit stress levels throughout the optical region of electromagnetic spectrum (400-2500 nm) (Fig. 1). At higher RWC, the reflectance values were lower in visible (400-700 nm) and SWIR (1100-2500 nm) regions due to absorption by pigments and water in leaves. As a result of water-deficit, reflectance in visible and SWIR regions increased owing to plant pigment degradation and lower leaf water content.

Variations in chlorophyll content

Genotypes exhibited considerable variations in their leaf chlorophyll contents. The leaf chlorophyll contents for genotypes are shown in Fig. 2. The summary statistics revealed that the chlorophyll content in the calibration data varied (Mean, SD) as 0.09-2.87 (1.96, 0.50), 0.17-1.37 (0.69, 0.28) and 0.27-3.99 (2.66, 0.71) mg g^{-1} FW for chlorophyll a, b and total content (Table 2). In validation subset, the chlorophyll content varied as 0.06-2.59 (1.96, 0.61), 0.13-1.34 (0.71, 0.29) and 0.19-3.90 (2.66, 0.80) mg g^{-1} FW for chlorophyll a, b and total chlorophyll content. This shows presence of subtle amount of variations in the data, which is favourable for developing good predictive models.

Relation between chlorophyll content and RWC

As the RWC decreased from 99 to 10%, the chlorophyll content also decreased and a power regressive trend was obtained. The relationship was good for total chlorophyll (R^2 = 0.67) and the poor in case of chlorophyll b (R^2 = 0.42) (Fig.

Table 1. Spectral indices for plant chlorophyll content estimation

Spectral indices	Defination	References
	Simple ratio indices	
Simple ratio pigment index	SRPI = R_{430}/R_{680}	Peñuelas *et al*. (1994)
Pigment-specific simple ratio-a	PSSRa = R_{800} /R_{680}	Blackburn (1998)
Pigment-specific simple ratio-b	PSSRb = R_{800}/R_{635}	
Pigment-specific simple ratio-c	PSSRc = R_{800}/R_{470}	
Buschman and Nagel index	R_{550} /R_{800}	Buschman and Nagel (1993)
Gitelson and Merzlyak index-1	GMI-1 = R_{750}/R_{550}	
Gitelson and Merzlyak index-2	GMI-2 = R_{750}/R_{700}	Gitelson and Merzlyak (1998)
Simple ratio (533, 565)	SR (533, 565) = R_{533}/R_{565}	Tian*et al*. (2011)
Vogelman index a	VOGa = R_{740}/R_{720}	Vogelman *et al*. (1993)
Zarco and Miller	ZM = R_{750}/R_{710}	Zarco-Tajada *et al*. (2001)
Ratio index-half	RI-half = R_{747}/R_{708}	Gupta *et al*. (2003)
Ratio index-1dB	RI-1dB = R_{735}/R_{720}	
Ratio index-2dB	RI-2dB = R_{738}/R_{720}	
Ratio index-3dB	RI-3dB = R_{741}/R_{717}	
	Normalized indices	
Normalized pigment chlorophyll index	NPCI = $(R_{680} – R_{430})/(R_{680} + R_{430})$	Penuelas *et al*. (1994)
Normalized difference vegetation index	NDVI = $(R_{830} – R_{670})$ /$(R_{830} + R_{670})$	Rouse et al.(1974)
Normalized phaeophytinization index	NPQI = $(R_{415} – R_{435})/(R_{415} + R_{435})$	Penuelas *et al*. (1995)
Pigment-specific normalized difference a	PSNDa = $(R_{800} – R_{680})/(R_{800} + R_{680})$	Blackburn(1998)
Pigment-specific normalized difference b	PSNDb = $(R_{800} – R_{635})/(R_{800} + R_{635})$	
Pigment-specific normalized difference c	PSNDc = $(R_{800} – R_{470})/(R_{800} + R_{470})$	
Normalized difference chlorophyll index	NDCI = $(R_{762} – R_{527})/(R_{762} + R_{527})$	Marshak *et al*. (2000)
Green normalized difference vegetation index	GNDVI = $(R_{750} – R_{550})/(R_{750} + R_{550})$	Gitelson *et al*.(1996)
Vogelman index b	VOGb = $(R_{734} – R_{747})/(R_{715} + R_{726})$	Zarco-Tejada *et al*. (2001)
Vogelman index c	VOGb = $(R_{734}– R_{747})/(R_{715} + R_{720})$	
Normalized difference red edge	NDRE = $(R_{790} – R_{720})/(R_{790} + R_{720})$	Rodriguez *et al*.(2006)
Photochemical reflectance index	PRI = $(R_{570} – R_{531})/(R_{570} + R_{531})$	Penuelas *et al*. (1995)
	Three-band indices	
Structure insensitive pigment index	SIPI = $(R_{800} – R_{445})/(R_{800} – R_{680})$	Penuelas *et al*. (1995)
Modified simple ratio$_{705}$	mSR705 = $(R_{750} – R_{445})/(R_{705} – R_{445})$	Sims and Gamon (2002)
Modified normalized difference$_{705}$	mND705 = $(R_{750} – R_{705})/(R_{750} + 2R_{445})$	Datt (1999)
Medium Resolution Imaging Spectrometer (MERIS) terrestrial chlorophyll index	MTCI = $(R_{750} – R_{710})/(R_{710} – R_{680})$	Dash and Curran (2004)

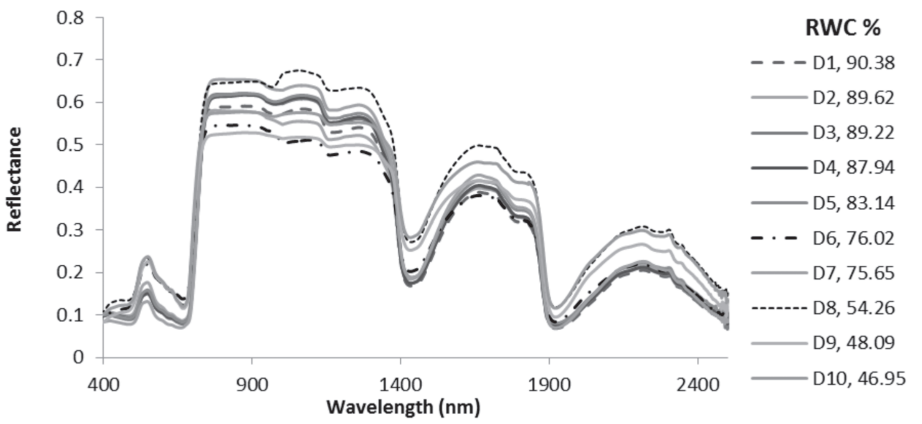


Fig. 1. Spectral reflectance variations of wheat crop at different water-deficit stress levels

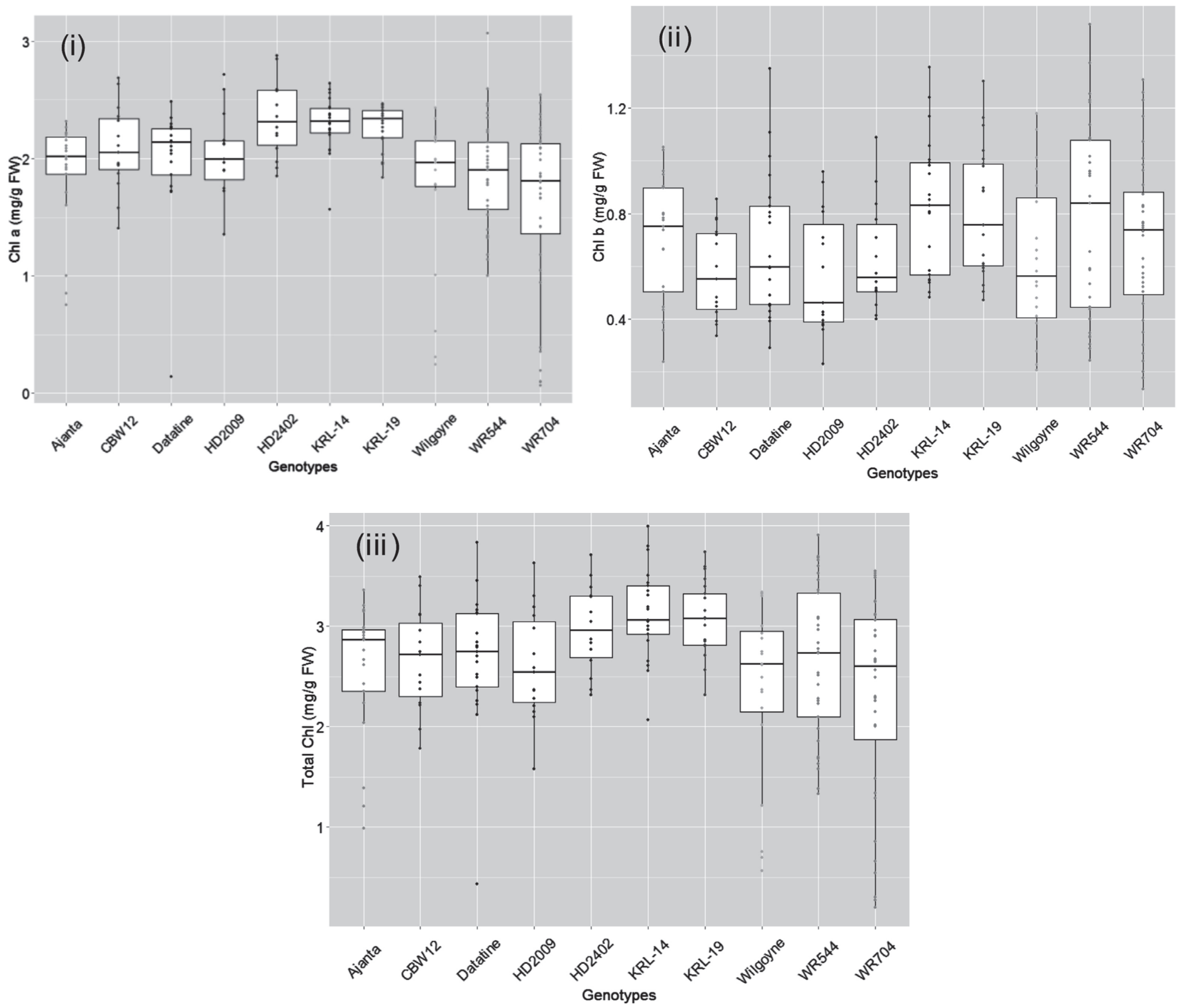


Fig. 2. Box plots showing the spreads of chlorophyll-a (i), chlorophyll-b (ii) and chlorophyll-total (ii) contents in different wheat genotypes.

Table 2. Summary statistics of chlorophyll content of calibration and validation datasets

	Sample size	Mean	Standard deviation	Minimum	Maximum	Skewness	Kurtosis	Standard error
				Chlorophyll a				
Calibration	145	1.96	0.50	0.09	2.87	-1.42	2.74	0.04
Validation	72	1.96	0.61	0.06	2.59	-1.69	2.61	0.07
				Chlorophyll b				
Calibration	145	0.69	0.28	0.17	1.37	0.31	-0.74	0.02
Validation	72	0.71	0.29	0.13	1.34	0.40	-0.36	0.03
				Total Chlorophyll				
Calibration	145	2.66	0.71	0.27	3.99	-0.98	1.17	0.05
Validation	72	2.66	0.80	0.19	3.90	-1.32	1.46	0.09

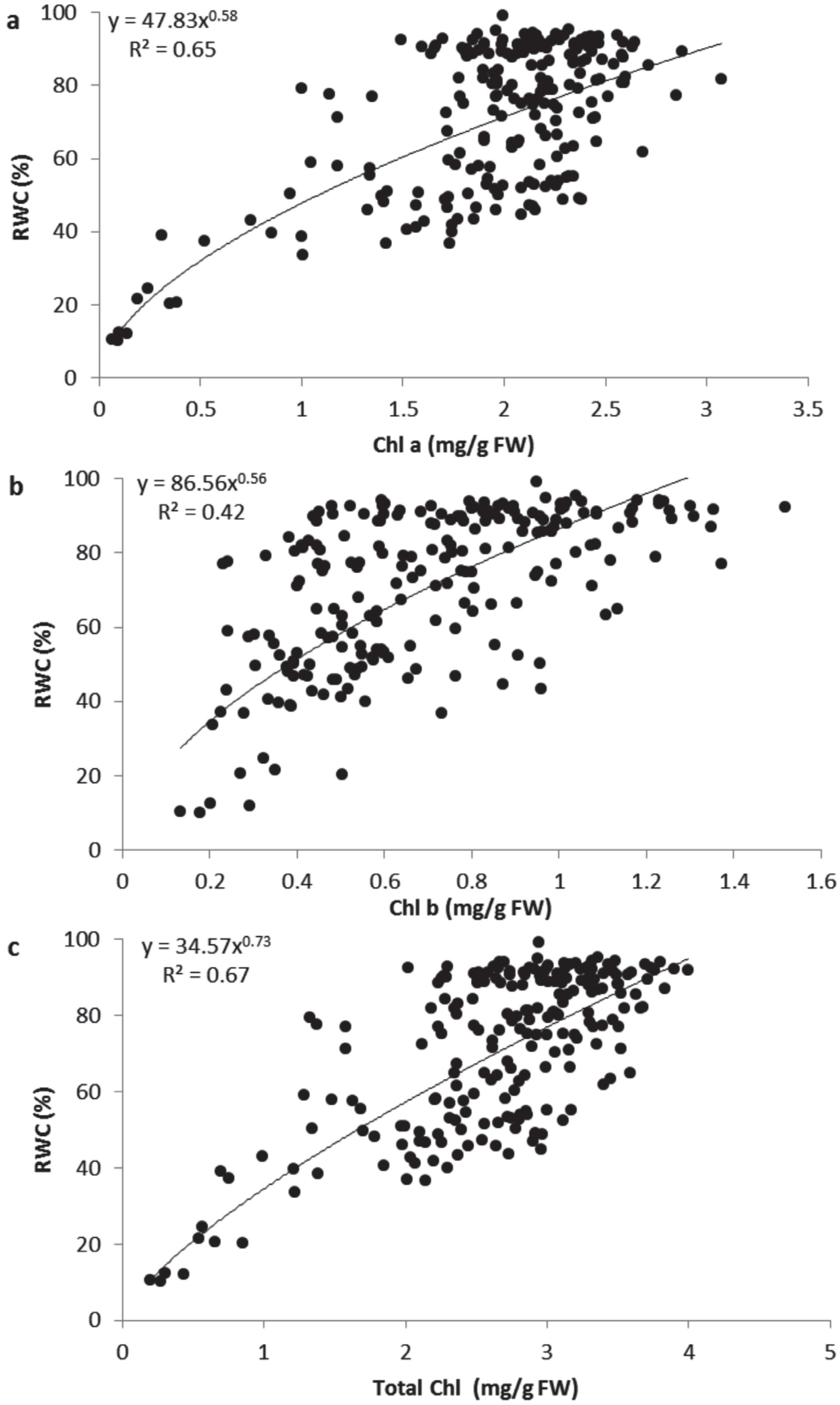


Fig. 3. Relationship of relative water content with (a) chl a (b) Chl b and (c) total chlorophyll content

3). A decrease in chlorophyll content with increase in water-deficit stress is in agreement with others (e.g. Fotovat *et al*., 2007; Arjenaki *et al*., 2012), but differs with a few (e.g. Schlemmer *et al*., 2005; Mensah *et al*., 2006). Water-deficit destroys the chlorophyll and inhibits further development (Lessani and Mojtahedi, 2002). Under water stress, plant produces reactive oxygen species (ROS) such as O^{2-} and H_2O_2 which can lead to lipid peroxidation and consequently, destruction of chlorophyll (Mirnoff, 1993; Foyer *et al*., 1994). Decreasing chlorophyll content may also act as a protection mechanism of plants under water-deficit stress by changing green leaf color into yellow, and thereby allowing more reflectance of the incident radiation (Schlemmer *et al*., 2005).

Correlation of chlorophyll content with spectral reflectance

Pattern of correlation coefficients between chlorophyll a and spectral reflectance at different hyperspectral bands was almost similar to that of chlorophyll b and total chlorophyll content (Fig. 4). Chlorophyll a, b and total chlorophyll contents were negatively correlated in visible region (400–

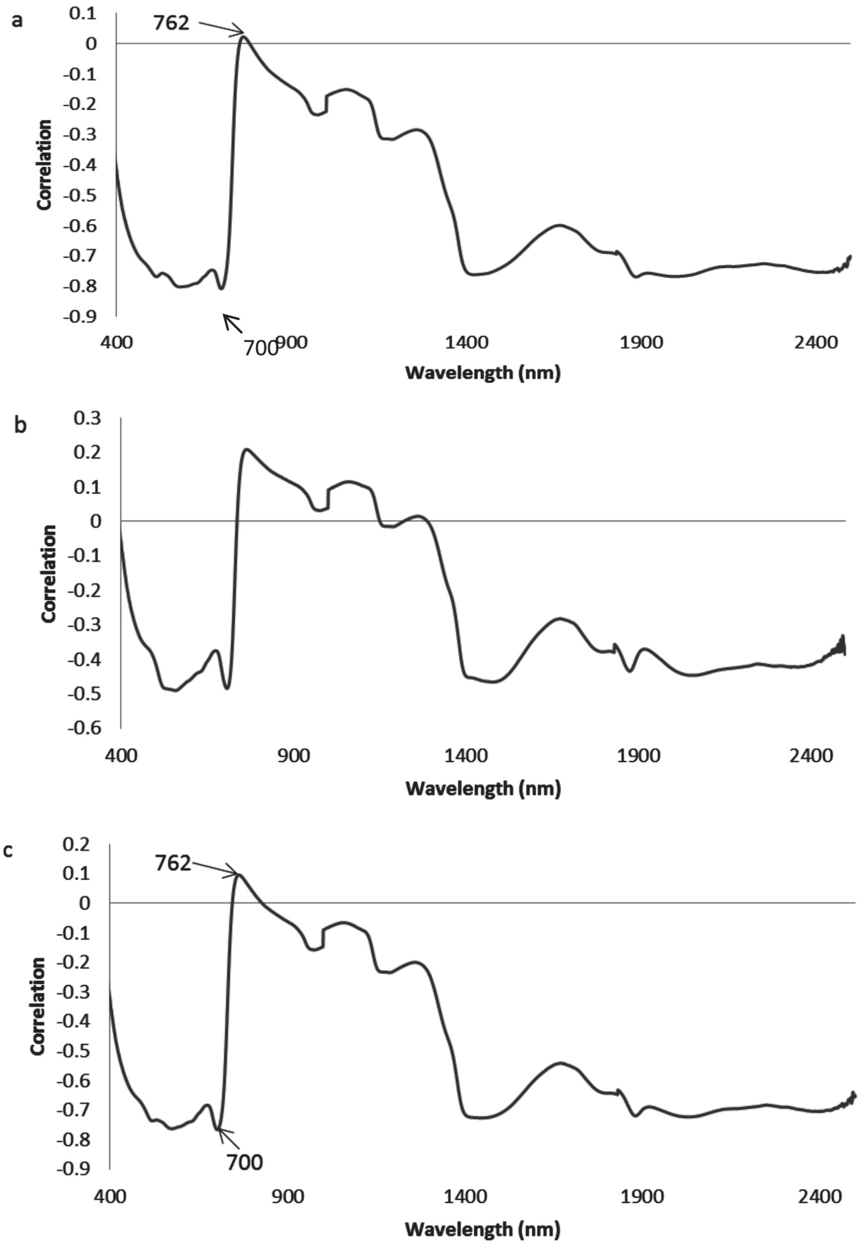


Fig. 4. Correlation of (a) chlorophyll a, (b) chlorophyll b and (c) total chlorophyll with reflectance spectra

725 nm), with chlorophyll a and total chlorophyll showing the highest correlation (-0.81 and -0.7, respectively at 700 nm). Chlorophyll b had the highest correlation (-0.49) at 660 nm. Positive correlations were obtained in NIR region for chlorophyll a at 737-1150 nm, chlorophyll b at 1219-1288 nm and total chlorophyll at 745-828 nm. The highest negative correlation at 600–705 nm bands for the chlorophyll was due to the strong chlorophyll absorption that reduced the reflectance. The indices were formulated by combining the sensitive wavelengths with maximum and minimum correlations identified in linear correlation analysis using the normalized structure. The indices in normalized form are given below:

$$NDSI_{Chla} = \frac{(R_{762} - R_{700})}{(R_{762} + R_{700})} \quad \ldots(5)$$

$$NDSI_{Chlb} = \frac{(R_{764} - R_{660})}{(R_{764} + R_{660})} \quad \ldots(6)$$

$$NDSI_{Total\ Chl} = \frac{(R_{762} - R_{700})}{(R_{762} + R_{700})} \quad \ldots(7)$$

The normalized structures of reflectance at wavelengths of interest were adopted to minimize the environmental interference in predictions of chlorophyll content in quantitative terms.

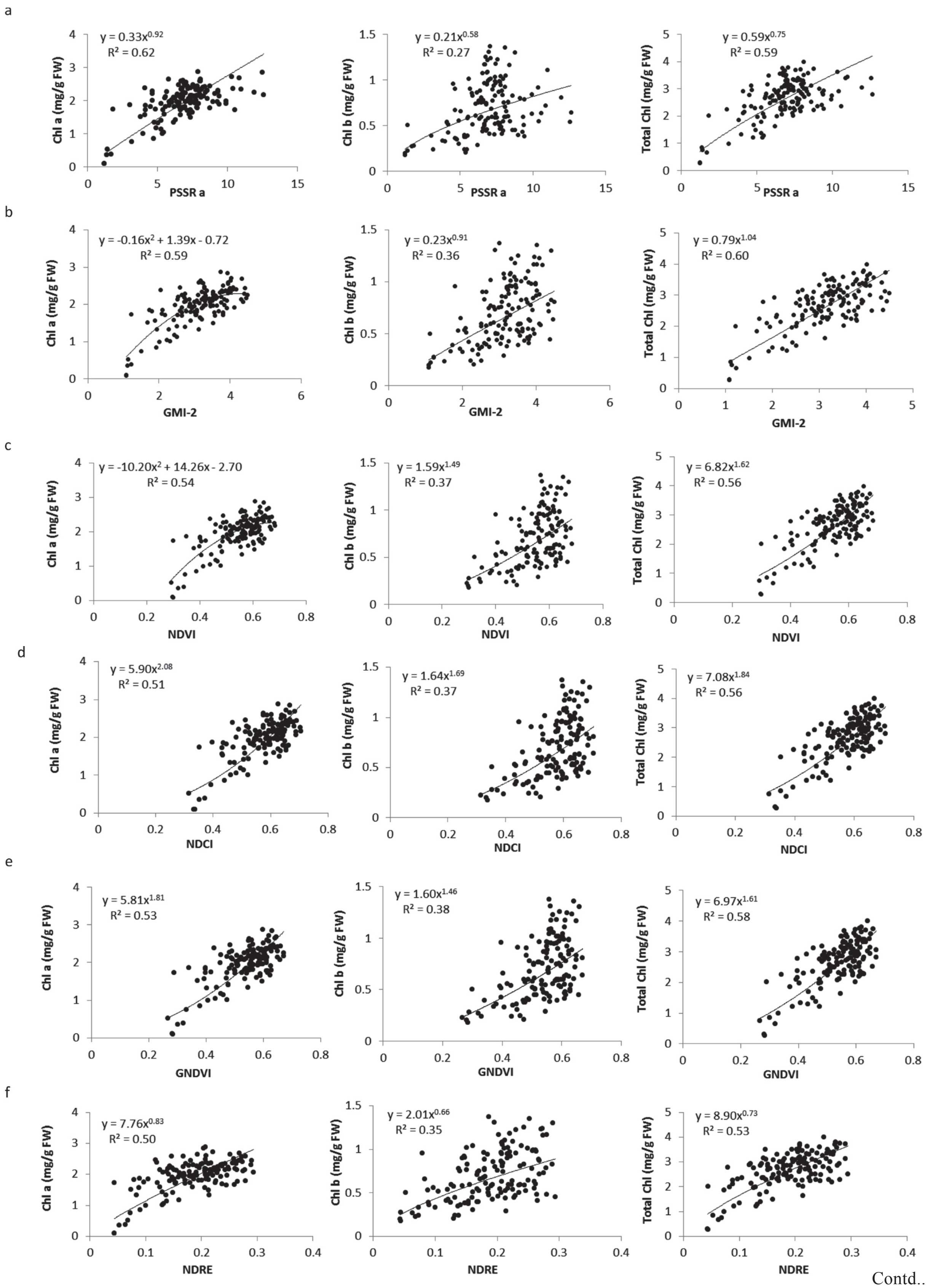
Spectral indices for characterization of chlorophyll content

The spectral indices based on reflectance in visible and NIR regions are sensitive to the leaf chlorophyll content. Thirty hyperspectral indices, which have been reported in the literature and are sensitive to plant chlorophyll content, were evaluated through the significance of their correlations with the a, b and total chlorophyll (Table 2). Correlations between indices and chlorophyll contents show medium to good R^2 values for chlorophyll a and total chlorophyll, but poor to medium for chlorophyll b (Table 3).

Among all the indices, the top nine indices with respect to R^2 were the pigment-specific simple ratio-a (PSSR-a), Gitelson and Merzlyak index-2 (GMI-2), normalized difference vegetation index (NDVI), normalized difference chlorophyll index (NDCI), green normalized difference vegetation index (GNDVI), normalized difference red edge (NDRE), modified simple ratio$_{705}$ (mSR705), modified normalized difference 705 (mND705) and Medium Resolution Imaging Spectrometer (MERIS) terrestrial chlorophyll index (MTCI). All these indices were related to chlorophyll content as power-fitted curve, except GMI-2, NDVI and mSR705 for chlorophyll a and mND705 for chlorophyll b (Fig. 5).

Table 3. Relationships of chlorophyll content to available spectral indices

Hyperspectral indices	R^2		
	Chl a	Chl b	Total Chl
SRPI	0.50	0.28	0.53
PSSRa	0.62	0.27	0.59
PSSRb	0.58	0.33	0.59
PSSRc	0.43	0.20	0.42
Buschman and Nagel index	0.53	0.28	0.52
GMI-1	0.46	0.26	0.47
GMI-2	0.59	0.36	0.60
SR (533, 565)	0.58	0.18	0.50
VOGa	0.44	0.29	0.47
ZM	0.51	0.33	0.54
RI-half	0.49	0.29	0.50
RI-1dB	0.46	0.30	0.48
RI-2dB	0.45	0.30	0.48
RI-3dB	0.46	0.29	0.48
NPCI	0.54	0.20	0.49
NDVI	0.54	0.37	0.56
NPQI	0.29	0.27	0.35
PSNDa	0.53	0.16	0.52
PSNDb	0.44	0.15	0.52
PSNDc	0.44	0.15	0.39
NDCI	0.51	0.37	0.56
GNDVI	0.53	0.38	0.58
VOGb	0.38	0.26	0.41
VOGc	0.37	0.26	0.40
NDRE	0.50	0.35	0.53
PRI	0.44	0.21	0.44
SIPI	0.39	0.09	0.32
mSR_{705}	0.57	0.43	0.58
mND_{705}	0.75	0.38	0.71
MTCI	0.70	0.35	0.68


a
y = 0.33x^0.92
R² = 0.62
Chl a (mg/g FW)
PSSR a
y = 0.21x^0.58
R² = 0.27
Chl b (mg/g FW)
PSSR a
y = 0.59x^0.75
R² = 0.59
Total Chl (mg/g FW)
PSSR a
b
y = -0.16x² + 1.39x - 0.72
R² = 0.59
Chl a (mg/g FW)
GMI-2
y = 0.23x^0.91
R² = 0.36
Chl b (mg/g FW)
GMI-2
y = 0.79x^1.04
R² = 0.60
Total Chl (mg/g FW)
GMI-2
c
y = -10.20x² + 14.26x - 2.70
R² = 0.54
Chl a (mg/g FW)
NDVI
y = 1.59x^1.49
R² = 0.37
Chl b (mg/g FW)
NDVI
y = 6.82x^1.62
R² = 0.56
Total Chl (mg/g FW)
NDVI
d
y = 5.90x^2.08
R² = 0.51
Chl a (mg/g FW)
NDCI
y = 1.64x^1.69
R² = 0.37
Chl b (mg/g FW)
NDCI
y = 7.08x^1.84
R² = 0.56
Total Chl (mg/g FW)
NDCI
e
y = 5.81x^1.81
R² = 0.53
Chl a (mg/g FW)
GNDVI
y = 1.60x^1.46
R² = 0.38
Chl b (mg/g FW)
GNDVI
y = 6.97x^1.61
R² = 0.58
Total Chl (mg/g FW)
GNDVI
f
y = 7.76x^0.83
R² = 0.50
Chl a (mg/g FW)
NDRE
y = 2.01x^0.66
R² = 0.35
Chl b (mg/g FW)
NDRE
y = 8.90x^0.73
R² = 0.53
Total Chl (mg/g FW)
NDRE

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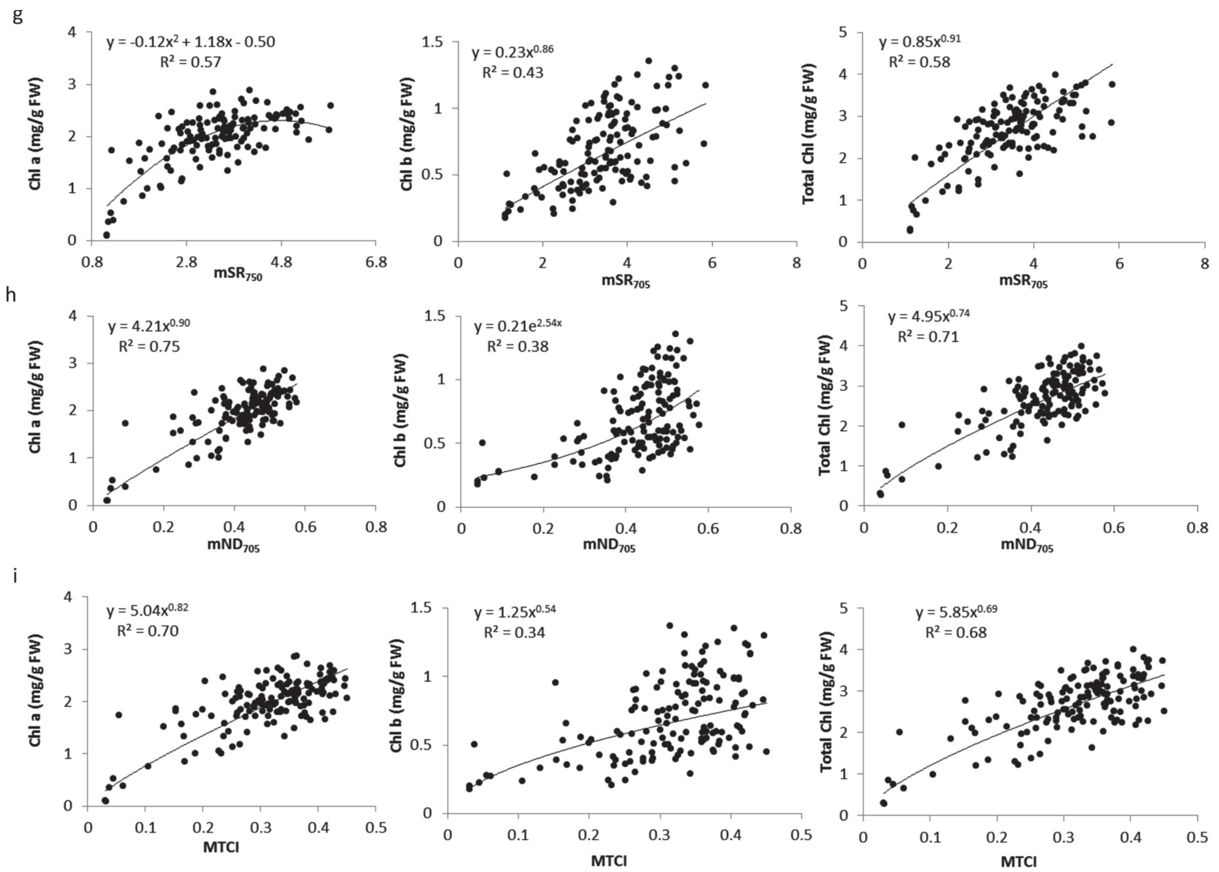
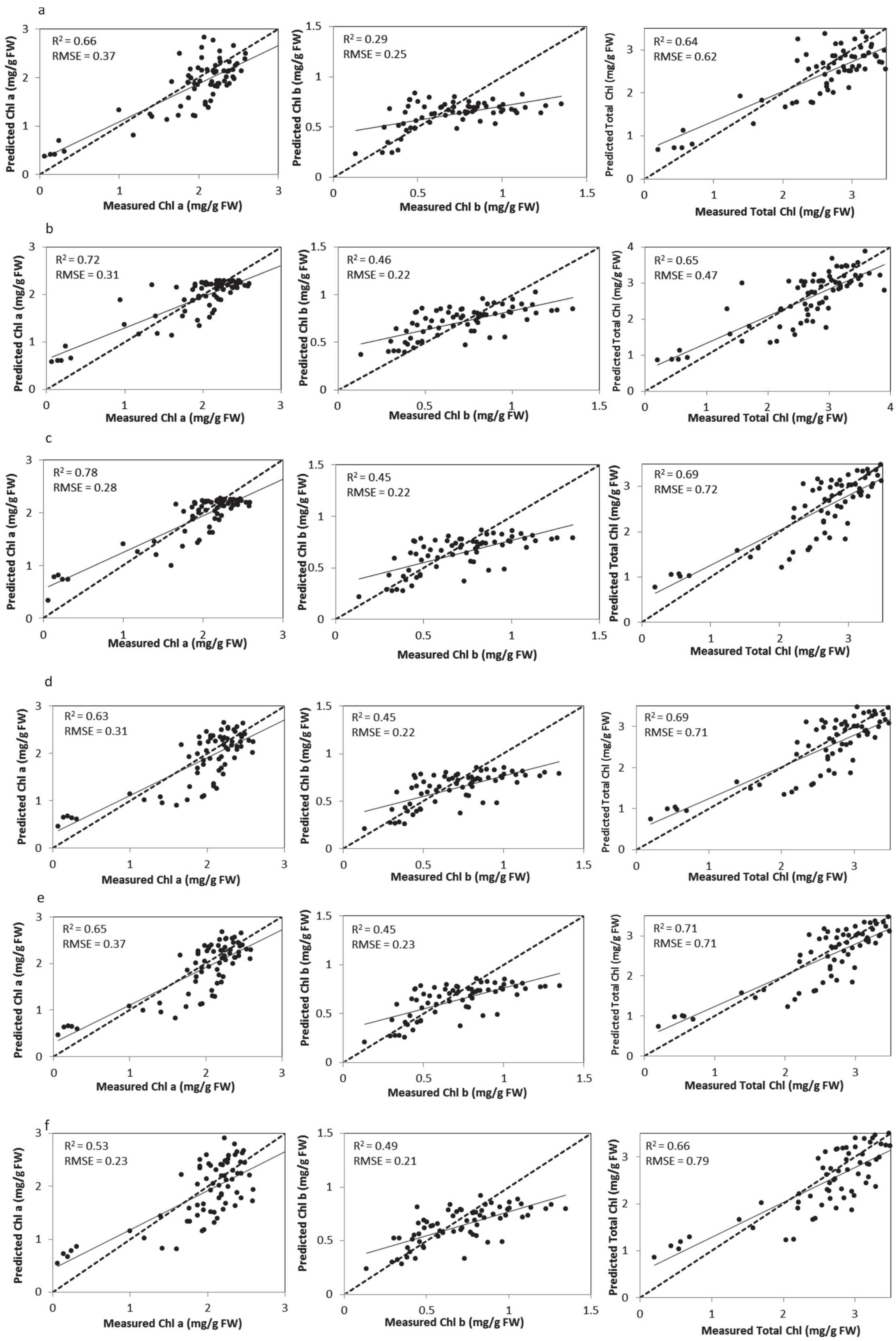


Fig. 5. Relation between chlorophyll content and (a) PSSR a (b) GMI-2 (c) NDVI (d) NDCI (e) GNDVI (f) NDRE (g) mSR705 (h) mND705 and (i) MTCI at water-deficit stress levels of wheat

Table 4. Comparative table of the nine best performing hyperspectral indices with newly proposed indices for leaf chlorophyll content estimation

Indices	Chl a			Chl b			Total Chl		
	R^2 (Cal)	R^2 (Val)	RMSE	R^2 (Cal)	R^2 (Val)	RMSE	R^2 (Cal)	R^2 (Val)	RMSE
PSSR a	0.62	0.66	0.37	0.27	0.29	0.25	0.59	0.64	0.62
GMI-2	0.59	0.72	0.31	0.36	0.46	0.22	0.60	0.65	0.47
NDVI	0.54	0.78	0.28	0.37	0.45	0.22	0.56	0.69	0.72
NDCI	0.51	0.63	0.31	0.37	0.45	0.22	0.56	0.69	0.71
GNDVI	0.53	0.65	0.37	0.38	0.45	0.23	0.58	0.71	0.71
NDRE	0.50	0.53	0.23	0.35	0.49	0.21	0.53	0.66	0.79
mSR_{705}	0.57	0.67	0.31	0.43	0.49	0.22	0.58	0.70	0.43
mND_{705}	0.75	0.76	0.30	0.38	0.44	0.21	0.71	0.73	0.43
MTCI	0.70	0.71	0.33	0.34	0.46	0.22	0.68	0.78	0.69
NDSI_Chla	0.83	0.79	0.29	-	-	-	-	-	-
NDSI_Total_Chl	-	-	-	-	-	-	0.76	0.81	0.38


a
R² = 0.66
RMSE = 0.37
R² = 0.29
RMSE = 0.25
R² = 0.64
RMSE = 0.62
b
R² = 0.72
RMSE = 0.31
R² = 0.46
RMSE = 0.22
R² = 0.65
RMSE = 0.47
c
R² = 0.78
RMSE = 0.28
R² = 0.45
RMSE = 0.22
R² = 0.69
RMSE = 0.72
d
R² = 0.63
RMSE = 0.31
R² = 0.45
RMSE = 0.22
R² = 0.69
RMSE = 0.71
e
R² = 0.65
RMSE = 0.37
R² = 0.45
RMSE = 0.23
R² = 0.71
RMSE = 0.71
f
R² = 0.53
RMSE = 0.23
R² = 0.49
RMSE = 0.21
R² = 0.66
RMSE = 0.79
Predicted Chl a (mg/g FW)
Measured Chl a (mg/g FW)
Predicted Chl b (mg/g FW)
Measured Chl b (mg/g FW)
Predicted Total Chl (mg/g FW)
Measured Total Chl (mg/g FW)

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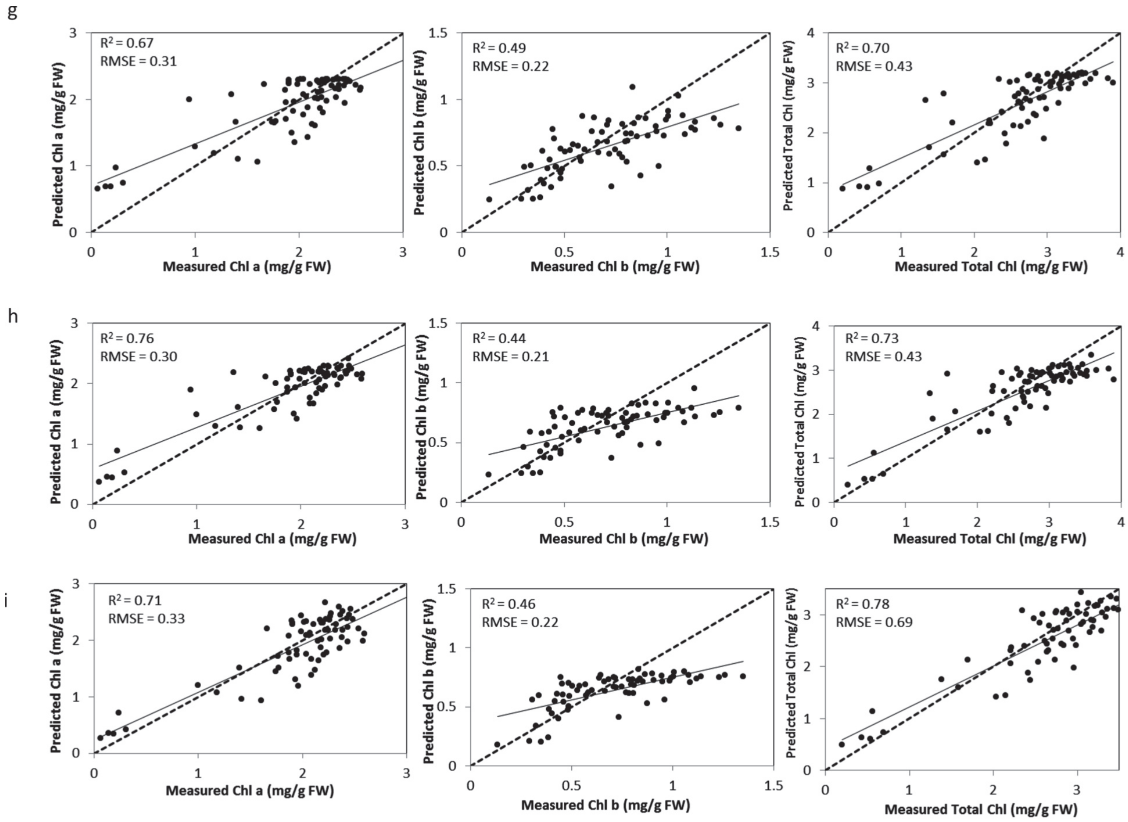

Fig. 6. Validation of the observed and predicted chlorophyll content from the indices and (a) PSSR a (b) GMI-2 (c) NDVI (d) NDCI (e) GNDVI (f) NDRE (g) mSR705 (h) mND705 and (i) MTCI at water-deficit stress levels of wheat

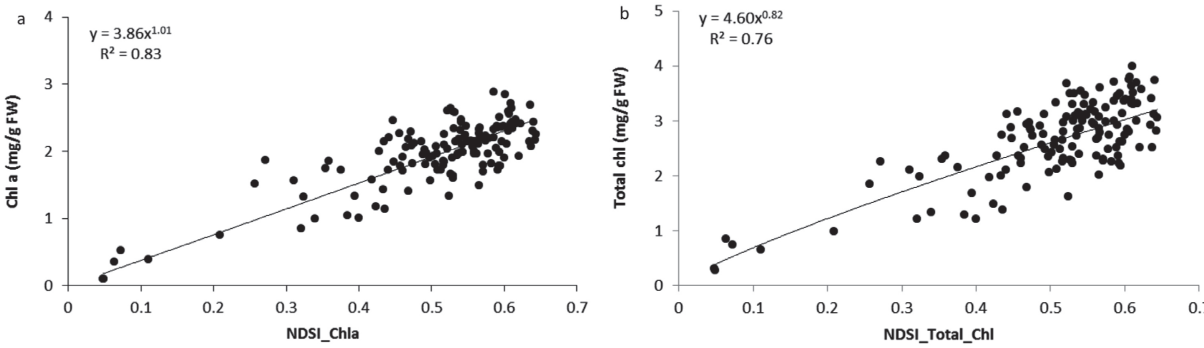

Fig. 7. Regression model for chlorophyll a (a) and total chlorophyll (b) content based on calibration dataset

The predicted chlorophyll content for nine selected indices shows moderate to good agreements with observed values in case of chlorophyll a and total chlorophyll but poor to moderate agreement for chlorophyll b (Fig. 6). New proposed normalized indices for chlorophyll a and total chlorophyll (NDSI_Chla (R_{700}, R_{762}), and NDSI_Total_Chl (R_{700}, R_{762}) were also calibrated and validated (Fig. 7, 8). Their performance was superior compared to existing indices (R^2 was 0.79 and 0.81, RMSE 0.29 and 0.38 mg g^{-1} FW for chlorophyll a and total chlorophyll) (Table 4). Again, chlorophyll b was in good relation (Data not presented). These new proposed spectral indices appear reliable and precise, and may be used for estimation of the chlorophyll content in wheat.

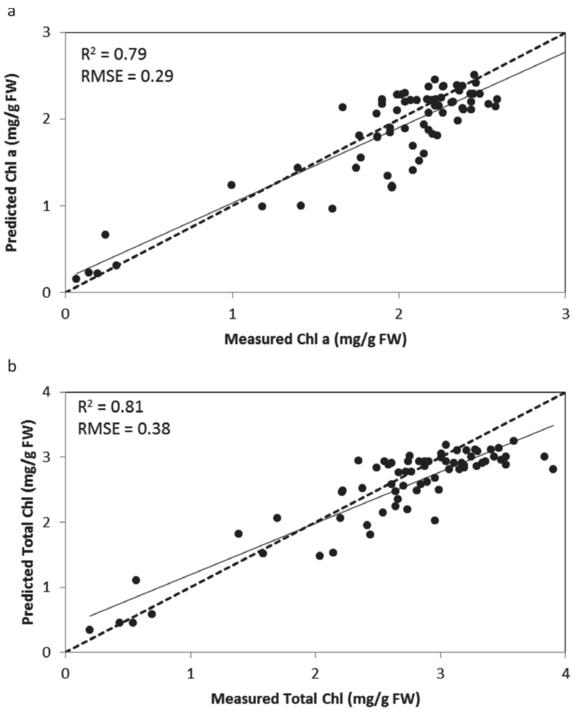

Fig. 8. Relationship between measured and predicted chlorophyll-a (a) and total chlorophyll (b) (mg g^{-1} FW) based on the developed model [Solid line is the regression, and the dotted is 1:1 line]

Conclusions

The quantitative relationship between leaf spectral reflectance and chlorophyll content was satisfactory developed at different water-deficit stress levels. Sensitive wavelengths to chlorophyll content in wheat were identified, and regression models were developed to retrieve chlorophyll a and total chlorophyll content in leaves with sufficient accuracy.

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