NDVI as a Potential Tool for Predicting Biomass, Plant Nitrogen Content and Growth in Wheat Genotypes Subjected to Different Water and Nitrogen Conditions

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The application of spectroradiometric index such as the normalized difference vegetation index (NDVI) to assess green biomass or nitrogen (N) content has focused on the plant canopy in precision agriculture or breeding programs. However, little is known about the usefulness of these techniques in isolated plants. The few reports available propose the use of a spectroradiometer in combination with special adaptors that improve signal acquisition from plants, but this makes measurements relatively slow and unsuitable. Here we studied the direct use (i.e. without adaptors) of a commercial cost-effective spectroradiometer, GreenSeeker^{IM} (NTech Industries Ins., Ukiah, California, USA) provided with an active sensor (i.e. equipped with its own source of radiation) for measuring NDVI in four genotypes of durum wheat (Triticum turgidum L. var. durum) grown in pots under a range of water and N regimes. Strong correlations were observed between NDVI measurements and dry aboveground biomass (AB), total green area (TGA), green area without spikes (GA) and aboveground N content (AN). To prove the predictive ability of NDVI measured under potted conditions, linear regression models for each growth trait and for plant N content were built with the data of two genotypes. The models accurately predicted growth traits and N content, confirming the direct relationship between total plant biomass and spectroradiometric readings.

Keywords: spectroradiometer, NDVI, active sensors, biomass, nitrogen content, green area, wheat

Abbreviations: TGA, total green area per plant; GA, green area without spikes; NDVI, normalized difference vegetation index; RMSE, root mean square error; RE, relative error; AB, aboveground biomass; AN, aboveground nitrogen content

Introduction

Spectroradiometric techniques in agriculture were initially developed to evaluate biomass and nitrogen (N) status at canopy level in order to support crop management. More recently, multispectral ground-based portable spectroradiometric devices had been used to

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characterize phenotypes in plant breeding (Araus 1996, 2001; Aparicio et al. 2000, Babar et al. 2006). Indeed, spectroradiometric vegetation index provide fast and non-destructive estimations of green biomass, chlorophyll content, leaf area index and grain yield at canopy level (Araus 1996; Aparicio et al. 2000; Bort et al. 2002). However, spectroradiometers have not been extensively used in agriculture because of their high cost. Moreover, most of them have passive sensors: i.e. they do not have their own light source and depend on an external source, such as sunlight. Consequently, spectroradiometric measurements depend on the weather conditions and daily changes in solar and sensor angle, as well as in background (Aparicio et al. 2004).

Spectroradiometric techniques may also be useful in breeding programs in which large numbers of genotypes (frequently thousands) under a range of environmental conditions, grown as isolated plants or potted plants, need to be screened (Alvaro et al. 2007). These techniques, providing a fast and non-destructive evaluation of plant traits, can be used as indirect breeding selection methods. Nevertheless, few studies have addressed the use of vegetation index to evaluate green biomass and/or N content of plants growing in pots or isolated in the field. In such studies plants were measured using an adaptor consisting of a cylinder with its own light source and reflecting walls (Casadesús et al. 2000; Alvaro et al. 2007). However, this kind of approach is not feasible for routine evaluation purposes owing to the time and human resources needed to perform such measurements (e.g. Alvaro et al. (2007) state that each single measurement takes at least 5 minutes and two people working together).

Recently, cost-effective and easy-to-handle spectroradiometers, such as the GreenSeekerTM (NTech Industries Inc., Ukiah, California, USA) equipped with an active sensor (i.e. with their own light source), have become available. The GreenSeekerTM is an integrated optical sensor that uses light emitting diodes (LED) to generate red and near-infrared (NIR) light, that measures the normalized difference vegetation index (NDVI, Rouse et al. 1973), computed as the normalized differences between NIR and red reflectance $[NDVI = (R_{NIR}-R_{red}) / (R_{NIR}+R_{red})]$, where R_{NIR} and R_{red} are the reflectance measured in the NIR (770 nm ë) and the red region (660 nm ë), respectively (Tremblay et al. 2009). NDVI value varies with absorption of red light by plant chlorophyll and the reflection of NIR radiation by water-filled leaf cells (Verhuslt and Govaerts, 2010). NDVI is very useful because it correlates positively with intercepted photo-synthetically active radiation and also correlates well with N content (Chen 1998). The NDVI measurements using a GreenSeekerTM had been used to assess grain yield in field canopies (Marti et al. 2007) and N status assessment of wheat (Filella et al. 1995; Li et al. 2008). In this study, the capacity of the NDVI was tested, measured directly (i.e. without any adaptor) by GreenSeekerTM, to assess plant growth and N content in contrasting genotypes of potted wheat grown under a range of water and N availability conditions.

Materials and Methods

Plant material and growth conditions

Four durum wheat (Triticum turgidum L. var. durum) genotypes, including three advanced lines (Bicrecham-1, Lahn/Haucan and Omrabi-3), released by the CIMMYT/ICARDA durum wheat breeding program, and one of the most frequently cultivated Spanish varieties (Mexa), were grown in a greenhouse at the Experimental Field Facilities of the University of Barcelona as previously described in Cabrera-Bosquet et al. (2007, 2009). Briefly, seven plants per pot were grown in cylindrical 5 L pots filled with washed sand in order to simulate an approximate real-field plant density (ca. 230 plants m^{-2}). Three different water regimes and two N levels (low and high) were imposed, by applying nutrient solution every 2 days. The three water regimes were well-watered (WW), intermediate (IS) and severe water stress (SS), respectively. The SS and IS water treatments were maintained at 40 and 70% of their container capacity, respectively, whereas the WW treatments involved full irrigation. The N supply was controlled by two different nutrient solutions: complete Hoagland solution (Hoagland and Arnon, 1950) and the same solution with N diluted four times (i.e. 0.06725 g N L⁻¹ and 0.27 g N L⁻¹ for low and high N, respectively). Different water and N treatments were used to create a broad range of plant response and explore the predictive capacity of NDVI at different levels of biomass. Plants were grown in a greenhouse under mean day/night temperatures of about 25/15°C and a maximum photosynthetic photon flux density (PPFD) of approximately 1,000 µmol $m^{-2} s^{-1}$. The experiment, consisting of 24 treatments (4 genotypes \times 3 water regimes \times 2 N levels), was replicated four times, thereby giving a total of 96 pots. Pots were arranged in a randomised complete block design (RCBD).

NDVI, plant biomass and N content determination

The NDVI of the total plant biomass in the pot was measured at stem elongation $(NDVI_{early})$ and anthesis $(NDVI_{late})$ coinciding with Feekes growth stages 7.0 and 10.5, respectively (Large 1954), by means of a portable GreenSeekerTM spectroradiometer. The sensor head was placed 70 cm above the surface of the pot, covering the total area of the plant with about 100 NDVI measurements taken at each pot. The NDVI measurements were soil-adjusted by subtracting NDVI measurements taken in empty pots containing only sand from measurements in pots with plants. Total aboveground biomass (AB) was collected at Feekes growth stage 10.5. Then the total green area per plant (TGA), including spikes, leaves and stems, and the green area without spikes (GA) were measured by means of a leaf area meter (DIAS, Delta-T Devices, Cambridge, UK). Then, AB was oven-dried at 80°C for 48 h, and weighed and ground. For each pot, a 2 mg subsample of finely ground dry matter was weighed in tin cups and N concentration was then measured using an elemental analyzer (Roboprep-CN EA, Europa Scientific Limited, Crewe, Cheshire, UK). Total aboveground N (AN) was then calculated as the N content expressed as percentage of plant dry weight multiplied by the dry aboveground biomass.

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Statistical analysis

A three-way analysis of variance (ANOVA) was performed to calculate the effects of water, N and genotype on the parameters studied. Means were compared with Duncan's multiple range test (P < 0.05). Then, to evaluate the predictive ability of NDVI measured in potted plants, the data set was divided into two groups: one was used to develop a general regression model and the rest was employed to validate the developed model. To create the general regression model, the homogeneity of the regression models adjusted for a particular genotype was determined by comparing their residues ($\sum SSi =$ summation of the residual sums of squares for individual fits to each genotype) with the residues of the general regression model (SSc = residual sum of squares for the common fit for all genotypes), as described by Borel et al. (1997):

$$F = \frac{\left| \text{SSC} - \sum_{i=1}^{n} \text{SSi} \right| / ((n-1)k)}{\sum_{i=1}^{n} \text{SSi} / (\text{Ndata} - k)}$$
(1)

which follows Fisher's law with (n-1)k and (Ndata-k) degrees of freedom. Ndata is the total number of data points, n the number of individual regressions and k is the number of fitted parameters for each regression (2 for the linear function).

The predictive ability of the general models was evaluated by means of coefficient of determination (R^2), root mean square error (*RMSE*) and relative error (*RE*,%) in prediction. *RMSE* and *RE* were calculated as:

$$RMSE = \sqrt{\frac{1}{N} \sum_{1}^{N} (Y_{REF} - Y_{PRED})^{2}}$$
(2)

$$RE(\%) = \frac{100}{\overline{Y}} \sqrt{\frac{1}{N} \sum_{1}^{N} (Y_{REF} - Y_{PRED})^{2}}$$
(3)

where Y_{REF} , Y_{PRED} were the measured and predicted values and \overline{Y} was the mean of the measured values of studied variables, respectively, and *N* was the number of samples. The prediction is considered excellent if RE < 10%, good if 10–20%, fair if 20–30% and poor if >30% (Jamieson et al. 1991). Data were analyzed by the SPSS statistical package (SPSS Inc., Chicago, IL).

Results

Effects of water regime, N supply and genotype on growth traits, AN and NDVI and relationship between traits

Water regime (WR), N supply (NS) and genotype (G) significantly affected the parameters studied (AB, TGA, GA, AN and NDVI) with water regime produced the greatest effect (Tables 1 and 2). In addition, plant response to water supply varied under different N

conditions and the four genotypes responded differently to variations of water and N levels, as confirmed by the significance of two- and three-way interactions (Table 1). Average values of AB, TGA, GA and AN ranged from 4.14 to 55.87 g, from 0.14 to 1.6 m², from 0.07 to 1.25 m² and from 0.06 to 1.84 g, respectively. Values of the NDVI measured at Feekes 7.0 and 10.5 ranged from 0.01 to 0.57 and from 0.03 to 0.71, respectively (Table 2). Under LN, an exponential increase in dry aboveground biomass and green area was recorded as response to increased water supply (+48% and +177% for AB; +38% and 186% for TGA; +50% and 213% for GA). Under optimal nutrient availability (HN), the highest increments were obtained with the intermediate water supply (+72% and +50% for AB; +56% and 43% for TGA; +62% and 48% for GA). This confirmed the positive interaction between N and water availability, evident for all the growth traits, and that, under sub-optimal N (LN) conditions, more water is needed to optimize plant growth.

Table 1. Sum of squares (type III) of the analysis of variance for the studied parameters across the treatments (water regime (WR), N supply (NS) and genotype (G))

Source of variation	df	Al	В	TGA	GA	AN	NDVIlate	NDVIearly
WR	2	9623	***	7.37 ***	3.59 ***	8.09 ***	0.97 ***	0.72 ***
NS	1	1941	***	1.32 ***	0.78 ***	4.51 ***	0.29 ***	0.11 ***
G	3	1119	***	0.32 ***	0.96 ***	1.25 ***	0.32 ***	0.17 ***
$WR \times NS$	2	290	***	0.36 ***	0.12 **	0.31 ***	0.03 **	0.01 ***
$WR \times G$	6	444	***	0.03 ns	0.09 ns	0.31 ***	0.09 ***	0.06 *
$NS \times G$	3	203	***	0.12 ns	0.14 **	0.64 ***	0.04 **	0.06 **
$WR \times NS \times G$	6	86	ns	0.05 ns	0.01 ns	0.16 **	0.01 ns	0.05 ns
Residual	72	536		1.19	0.56	0.50	0.21	0.31
Total	95							

*P < 0.05, **P < 0.01, ***P < 0.001, ns Non significant

AB, aboveground biomass; TGA, total green area per plant; GA, total green area without spikes; AN, aboveground N content; NDVI, normalized difference vegetation index. NDVI_{early} and NDVI_{late} represent NDVI measured at Feekes 7 and Feekes 10.5, respectively.

The four genotypes also responded differently to N and water supply (significant interactions: NS × G for AB and GA; WR × G for AB; WR × NS × G for AN). Bicrecham-1 and Omrabi-3 showed the highest average increments in terms of AB to increased N supply (68 and 61%, respectively), although the former had lower average values that were more like Lahn/Haucan (16 and 27 g per plant for Bicrecham-1 compared to 20.7 and 33 g per plant for Omrabi-3, under LN and HN, respectively). Mexa showed the lowest response to N supply (35%). For GA, the highest and lowest increments were shown by Omrabi-3 and Mexa, respectively. To the increase in water availability, Lahn/Haucan and Omrabi-3 had the highest response in terms of AB, with the intermediate water supply. As concerns the total aboveground N content (AN), passing from SS to IS, Bicrecham-1 showed the greatest differences as function of N availability: AN increased by only 29% under LN, whereas it rose by 77% under HN. However, Lahn/Haucan and Omrabi-3 genotypes had more similar behaviour under different nutritional conditions: 83 and 88% under LN, 93

		Water Regime ¹		N Suj	ply ²		Genot	ype ³	
	SS	IS	WM	Low	High	Bicrecham-1	Lahn/Haucan	Mexa	Omrabi-3
AB (g DW)	$11.3\pm0.8^{\circ}$	$18.6\pm1.5^{\rm b}$	35.2 ± 1.4^{ac}	17.2±1.6 ^b	26.2 ± 1.7^{a}	$21.5\pm2.3^{\mathrm{b}}$	20.8±2.3 ^b	$17.5{\pm}1.8^{c}$	$27.0{\pm}3.1^{a}$
TGA (m ²)	$0.36{\pm}0.03^{\circ}$	$0.54{\pm}0.04^{ m b}$	$1.01{\pm}0.03^{a}$	$0.52{\pm}0.04^{\rm b}$	$0.75{\pm}0.05^{a}$	$0.63{\pm}0.04^{ m b}$	$0.62{\pm}0.07^{ m b}$	$0.57 {\pm} 0.06^{\rm b}$	$0.71{\pm}0.07^{\mathrm{a}}$
GA (m ²)	$0.22{\pm}0.02^{\circ}$	$0.34{\pm}0.03^{ m b}$	$0.67{\pm}0.03^{a}$	0.32 ± 0.03^{b}	$0.49{\pm}0.04^{a}$	$0.40{\pm}0.04^{ m b}$	$0.40{\pm}0.04^{ m b}$	0.29±0.03°	$0.55{\pm}0.06^{a}$
AN (g)	$0.31{\pm}0.03^{\circ}$	$0.54{\pm}0.06^{\mathrm{b}}$	$1.01{\pm}0.06^{a}$	$0.41{\pm}0.04^{\rm b}$	$0.84{\pm}0.06^{a}$	$0.65{\pm}0.08^{ m b}$	$0.60{\pm}0.07^{ m b}$	$0.46 \pm 0.05^{\circ}$	$0.78{\pm}0.10^{\mathrm{a}}$
NDVI _{carly}	$0.11\pm0.01^{\circ}$	$0.19{\pm}0.02^{b}$	$0.31{\pm}0.01^{a}$	0.17 ± 0.02^{b}	$0.23{\pm}0.02^{a}$	$0.20{\pm}0.02^{\rm b}$	$0.19{\pm}0.02^{\rm b}$	$0.15 \pm 0.02^{\circ}$	$0.27{\pm}0.03^{\rm a}$
NDVI _{late}	$0.17\pm0.01^{\circ}$	0.27 ± 0.02^{b}	$0.42{\pm}0.02^{a}$	0.23 ± 0.02^{b}	$0.34{\pm}0.02^{a}$	$0.28{\pm}0.03^{ m b}$	$0.28 \pm 0.03^{\rm b}$	0.22±0.02°	$0.38{\pm}0.04^{\mathrm{a}}$
AB, aboveground	d biomass; TGA,	, total green area	per plant; GA, t	total green area	without spikes;	AN, abovegroui	nd N content; ND	VI, normalized	difference
vegetation index.	. Data are the me	$\sin \pm SE \text{ of } 24^3$,	32 ¹ or 48 ² replic:	ates. Within eac	h row and each	treatment, value	s with different s	superscripted le	tters are signif-
icantly different :	according to Dur	ncan test ($P < 0.0$	15).						

Table 2. Main descriptive statistics for the studied parameters

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and 82% under HN conditions. Mexa had again the lowest response in both N conditions. In addition, both Bicrecham-1 and Omrabi-3 were the most responsive to N under SS conditions.

Therefore, Bicrecham-1 was more affected by low N conditions, as shown also by the low response to water under LN availability; Omrabi-3 had the higher response to both WR and NS. Mexa showed lower response to both factors. NDVI, whether measured at early or late growth stages, reflected the trends shown by the growth traits. Accordingly, Omrabi-3 and Mexa had the highest and the lowest NDVI values, whilst Bicrecham-1 and Lahn/Haucan showed intermediate NDVI values. Results showed that all growth traits (AB, TGA and GA) and AN significantly (P < 0.001) and positively correlated with NDVI measurements performed at both growth stages (early and late). However, these correlations considerably improved when the traits measured were compared with NDVI measurements performed closer to anthesis (NDVI_{late}) (Table 3).

<i>Table 3</i> . Linear regression equations between NDVI _{early} and NDVI _{late} , on one hand, and aboveground
biomass (AB), aboveground N content (AN), total green area (TGA) and total green area without spikes (GA).
on the other hand

	NDVI _{ear}	NDVI _{early}		NDVI _{late}	
	Regression equation	R^2	Regression equation	R^2	
Aboveground Biomass (AB	B)				
Bicrecham-1	y = 78.68x + 5.83	0.64	y = 87.83x-2.83	0.91	
Lahn/Haucan	y = 102.32x + 1.38	0.79	y = 83.08x-2.14	0.83	
Omrabi-3	y = 74.41x + 7.11	0.67	y = 81.17x - 3.57	0.91	
Mexa	y = 77.41x + 5.85	0.70	y = 87.83x - 1.49	0.87	
Aboveground Nitrogen (Al	N)				
Bicrecham-1	y = 2.40x + 0.17	0.47	y = 3.14x - 0.21	0.92	
Lahn/Haucan	y = 3.24x - 0.01	0.71	y = 2.70x - 0.14	0.79	
Omrabi-3	y = 2.44x + 0.13	0.65	y = 2.52x - 0.17	0.80	
Mexa	y = 2.08x + 0.14	0.62	y = 2.47x-0.07	0.84	
Total Green Area (TGA)					
Bicrecham-1	y = 2.64x + 0.11	0.73	y = 2.54x - 0.07	0.77	
Lahn/Haucan	y = 3.12x + 0.03	0.80	y = 2.46x - 0.06	0.81	
Omrabi-3	y = 1.64x + 0.30	0.59	y = 1.95x-0.01	0.89	
Mexa	y = 2.70x + 0.16	0.73	y = 2.92x - 0.06	0.82	
Green Area (GA)					
Bicrecham-1	y = 1.75x + 0.05	0.70	y = 1.76x-0.09	0.80	
Lahn/Haucan	y = 2.15x + 0.02	0.80	y = 1.71x-0.08	0.81	
Omrabi-3	y = 1.38x + 0.20	0.60	y = 1.63x - 0.04	0.89	
Mexa	y = 1.55x + 0.05	0.73	y = 1.67x-0.07	0.81	

All correlations were significant at $P \le 0.001$ level. NDVI_{early} and NDVI_{late} represent NDVI measured at Feekes 7 and Feekes 10.5, respectively.

General model construction and validation

Due to the similarity between Bicrecham-1 and Lahn/Haucan durum genotypes in terms of plant growth and plant N content (Table 2), a general regression model for each of the studied parameters against the NDVI_{late}, including data of the two selected genotypes, was developed. However, before the general regression models were constructed, the homogeneity of the regression coefficients of the different regressions between NDVI and the parameters studied for each genotype was tested. The NDVI values and measured traits for the two chosen genotypes were plotted together, and simple linear regressions were fitted between the NDVI and AB ($R^2 = 0.87$, P < 0.001), TGA ($R^2 = 0.77$, P < 0.001), GA ($R^2 = 0.82$, P < 0.001) as well as AN ($R^2 = 0.85$, P < 0.001) (Fig. 1). Each of the linear regression



Figure 1. Relationships between NDVI measurements performed at Feekes growth stage 10.5 (NDVI_{late}) and aboveground biomass (AB), total green area per plant (TGA), total green area without spikes (GA), aboveground N content (AN). Within each plot, circles represent data from Bicrecham-1 and Lahn/Haucan genotypes including all growth treatments (n = 48). Dashed lines represent the confidence intervals (P < 0.05)</p>

models (AB vs. NDVI, AN vs. NDVI, TGA vs. NDVI and GA vs. NDVI) was tested with data from Omrabi-3 (Fig. 2) and Mexa (Fig. 3) genotypes.



Figure 2. Relationships between the measured and predicted traits for Omrabi-3. Aboveground biomass (AB), aboveground N content (AN) total green area per plant (TGA) and total green area without spikes (GA), were predicted using linear regression models calibrated with data from Bicrecham-1 and Lahn/Haucan genotypes. The dashed lines represent a 1:1 correlation

The measured AB values compared satisfactorily with NDVI-predicted AB values. In Omrabi-3, the regression model consistently predicted the AB with R^2 of 0.92, *RMSE* of 4.8 g and *RE* of 18.4%, which is considered a good prediction according to the criterion of Jamieson et al. (1991). Similar results were found in Mexa with R^2 of 0.87, *RMSE* of 3.53 g and *RE* of 20.1%. In both cases, the slopes of the regression lines between predicted and measured AB values were close to 1. Fair results were found in the AN prediction in both genotypes. In Omrabi-3, although the regression between the measured and predicted AN values showed a good slope of 0.95 and R^2 of 0.86, *RMSE* of 0.23 g and *RE* of 29.3% weakened the prediction. Conversely, in Mexa, while the *RMSE* and the *RE* scored quite good values of 0.11 and 23.4%, respectively. The regression line showed a slope of 0.85 and R^2 of 0.84. The TGA and GA values were properly predicted by the models in both genotypes (Figs 2 and 3). However, predictive values of NDVI were higher for GA than



Figure 3. Relationships between the measured and predicted traits for Mexa. Aboveground biomass (AB), aboveground N content (AN) total green area per plant (TGA) and total green area without spikes (GA), were predicted using linear regression models calibrated with data from Bicrecham-1 and Lahn/Haucan genotypes. The dashed lines represent a 1:1 correlation

TGA. In Omrabi-3, predicted TGA values were generally higher than measured ones (slope of 0.81); while in Mexa, predicted TGA values were lower than measured ones (slope 1.17). The *RE* was 25.9 and 28.2% for Omrabi-3 and Mexa, respectively. Conversely, the regression found between the measured and predicted GA values showed a good fit in both genotypes, with slopes of almost 1 (0.94 and 0.96 for Omrabi-3 and Mexa, respectively), and *RMSE* and *RE* values of 0.10 and 17.9% in Omrabi-3 and 0.07 and 25.4% in Mexa.

Discussion

Effects of water regime, N supply and genotype on growth traits, AN and NDVI

The combined effects of WR, NS and G resulted in a wide range of plant response for the studied parameters, useful for evaluating the predictive ability of NDVI at different levels of biomass and green area produced. Bicrecham-1 and Lahn/Haucan showed similar char-

acteristics in terms of growth and plant N content; Omrabi-3 and Mexa showed the largest differences (Table 2) in such traits. Omrabi-3, a late-maturing cultivar, showed a higher plant growth, resulting in a greater biomass and development of leaf area (high TGA and GA); whereas Mexa, a Spanish commercial cultivar, showed the lowest biomass accumulation and, therefore, the lowest TGA and GA values (Cabrera-Bosquet et al. 2007, 2009).

Relationship between NDVI and measured traits

Linear regressions were found to be the simplest adjustment to fit the relationships between NDVI and the traits measured for each of the four genotypes (Table 3). However, exponential rather than linear relationships between NDVI and growth traits have been extensively reported (Gamon et al. 1995, Aparicio et al. 2000, Alvaro et al. 2007). This can be explained by the saturation pattern that occurs when LAI values > 2 and NDVI becomes insensitive to further increases in LAI (Gamon et al. 1995). At these LAI values, NDVI usually become greater than 0.5 (Aparicio et al. 2000). This was not the case in our study, in which, as NDVI values were mostly below 0.5, only the linear trait of the relationship was examined. Nevertheless, linear relationships between NDVI and biomass have been reported in wheat (Marti et al. 2007). In addition, the improved correlations between growth traits and NDVI measurements at late growth stage can be explained by that maximum biomass in cereals, which is reached around anthesis, correlates strongly with grain yield and is, therefore, a well-accepted trait for plant phenotyping in breeding programs (Richards et al. 2002). This also can be explained because growth traits measured and NDVI_{late} readings were carried out at the same growth stage (Feekes 10.5). Nevertheless, Marti et al. (2007), working with bread wheat in field plots, also found with a similar spectroradiometer increase in the correlations between growth and NDVI measurements performed around anthesis.

Model validation

Overall, prediction of AB proved better than of AN in both genotypes, which confirmed the strong relationship between total plant biomass (and therefore total plant chlorophyll) and spectroradiometric readings. This is based on the fact that NDVI measures the proportion between the light reflected by chlorophyll and the light reflected by other cell structures. Such a relationship has been reported extensively in wheat canopies (Bellairs et al. 1996; Araus et al. 2001; Aparicio et al. 2002). Correlations between NDVI and GA fitted better than those between NDVI and TGA. In addition, NDVI predictions of GA were generally more accurate than of TGA. Alvaro et al. (2007) found similar results in individual plants of various small grain cereals grown in the field, with an improvement in most correlations when only data from plants without spikes were analyzed. This pattern was probably related to the difficulties in accurately determining ear area (Alvaro et al. 2007). The models worked successfully with higher prediction accuracy of growth traits and N content in two genotypes that differed greatly in terms of plant growth and N accumulation.

In conclusion, the use of a portable active sensor spectroradiometer like the GreenSeekerTM was a useful tool for predicting growth traits and N content in two geno-

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types that differed greatly in terms of plant growth and N accumulation. In addition, instruments and the methodological procedure used to measure NDVI from plants grown in pots proved easier and more convenient than previous approaches (Casadesús et al. 2000; Alvaro et al. 2007), helping to reduce the amount of time spent on measurements. The method we proposed becomes appropriate for routine measurements in a large number of plants, and such an approach can be applied in field conditions and may be particularly useful in the initial stages of breeding programs, when plants usually grow in isolation and fast, easy and inexpensive screening measurements are desirable.

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