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EFFECT OF NITROGEN AND ENDOPHYTIC BACTERIA ON BIOPHYSICAL AND SPECTRAL PARAMETERS OF WHEAT CANOPY

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Abstract: Nitrogen (N) is the primary limiting factor to increase wheat (*Triticum aestivum* L.) yield in Brazil and the use of N fixing agents might be an alternative to increase wheat production. A field experiment was conducted in 2006 in São Paulo state, Brazil, with three N rates (0, 60 and 120 kg ha^{-1}) and three endophytic bacteria (IAC-HT-11; IAC-HT-8; and IAC-AT-8) (1) to evaluate their combined impact on biophysical (LAI and grain yield) and spectral parameters (NDVI, absorption band parameters) of wheat and (2) to determine the spectral parameters that best correlated with LAI and grain yield. LAI and canopy spectral reflectance measurements were made at jointing, heading and ripening growth stages for this purpose. The statistical relationships between biophysical and spectral parameters were evaluated within and among the treatments. Statistical results showed that the N rates significantly affected both biophysical and spectral parameters as expected. Bacteria inoculation presented a significant effect only on grain yield and is consistent with results reported in the literature. The NDVI followed by absorption band parameters P_B and A_B at 1205 nm provided best estimates of LAI and grain yield during the heading stage indicating that it may be the best stage for remote sensing based yield mapping.

Keywords: field spectroradiometry, remote sensing, reflectance, NDVI, LAI.

INTRODUCTION

Nitrogen (N) is considered as the primary limiting factor to increase wheat (*Triticum aestivum*

L.) yields in Brazil (Sala et al., 2005). In search for alternatives that would reduce this limitation, research has been conducted with endophytic bacteria and fungi as nitrogen fixing agents for wheat plant while the plant is furnishing energy for bacteria or fungi metabolism (Conn, 2005; Sala et al., 2005; Larran et al., 2007). The endophytic bacteria and fungi remain within the living plant tissue for part or all of their life cycle, increasing N availability without causing visible symptoms of infection (Conn and Franco, 2004; Kizilkaya, 2008). Rodrigues et al. (2000) showed an increase in N content in the grain with bacteria inoculation and no improvement in wheat yield. Sala et al. (2007) reported that wheat inoculated with nitrogen fixing bacteria with no additional N application increased biomass and grain yield. According to Freitas (2000), practical use of bacteria as inoculants still requires more experiments because their effects on yield and N assimilation of wheat are not well understood. Increase in N concentration of wheat produces spectral reflectance changes that may be detected by remote sensing instruments, as demonstrated by several researchers (Serrano et al. 2000; Mullen et al., 2003; Pena-Yewtukhiw et al., 2006; Shou et al., 2007; Sripada et al., 2007; Tilling et al., 2007; Feng et al., 2008). If bacteria treatment affects yield and N assimilation of wheat, it may affect their relationships with spectral parameters. However, bacteria inoculation effects on wheat reflectance have not been reported in the literature.

From a remote sensing perspective, plants absorb more solar energy in the visible region with increasing biomass. In the near-infrared region, they reflect and transmit more energy due to increase in the multiple scattering and transmission of radiation that occur on both individual leaves and the entire plant canopy (Goel, 1988). As a result, vegetation indices such as the normalized difference vegetation index (NDVI), the most frequently used spectral vegetation index, are usually correlated with leaf area index (LAI) and crop yield (Aparicio et al., 2000; Serrano et al., 2000; Thenkabail et al., 2000; Elwadie et al., 2005; Eitel et al., 2008; Raun et al., 2008; Galvão et al., 2009).

Advances in the hyperspectral remote sensing technology are driving the development of new analytical methods that consider not only the vegetation indices to estimate biophysical parameters, but also the continuous and individual features of reflectance spectra (Meer, 2000; Wiegand et al., 2001). For example, absorption bands associated with the chlorophyll (665 nm) and leaf water content (982 and 1205 nm) can be measured by laboratory, field, airborne or orbital non-imaging and imaging spectrometers. Such features can be extracted from spectra using the continuum removal method (Clark and Roush, 1984), which allows the subsequent determination of the depth (P_B) and area (A_B) of each absorption band (Meer, 2000; Mutanga et al., 2003). Leaf water absorption bands may also be correlated with LAI

(Gao, 1996) and crop yield (Galvão et al., 2009). Despite these advances most of the studies to determine biophysical parameters of wheat are still based on vegetation indices (e.g., Mullen et al., 2003; Wu et al., 2008). Only a few works have addressed spectral feature analysis for this purpose (Zhao et al., 2004; Delegido et al., 2008), and none of them has compared the performance of vegetation indices and absorption band parameters calculated from the continuum removal method to estimate LAI and yield.

The objectives of this field experiment were: (1) to evaluate the combined impact of three rates of N and three endophytic bacteria treatments on biophysical (LAI and grain yield) and spectral parameters (NDVI, absorption band parameters) of wheat (*Triticum aestivum* L.); and (2) to determine the spectral parameters that best correlated with LAI and grain yield.

METHODS

Experiment

This experiment was conducted at the research farm of the Agronomic Institute of Campinas located in the Campinas municipality, São Paulo State, Brazil (22° 51' 53" S and 47° 04' 52" W). Wheat plants were grown from June to September of 2006. According to the Köppen classification, the climate of this region is Cwa (humid subtropical with dry winter). During the growing season, the mean temperature was 18.4, 18.7, 20.5 and 20.9 ºC in June, July, August, and September, respectively. The monthly precipitation in June, July, August, and September was 22.5, 48.8, 12.4 and 101.9 mm, respectively. This pattern was close to normal conditions for this region. The average water demand for wheat is 4 mm per day; therefore, water was supplemented with irrigation. Canopy reflectance, LAI and yield data were collected for each wheat plot treated with different endophytic bacteria and N rates. The experimental design was a factorial randomized complete block with six replications of treatments that were applied to a single wheat cultivar (IAC-370 *Triticum aestivum* L.) and consisted of one control and three isolates of endophytic bacteria (B0 – without bacteria or control; B1 – IAC-HT-11 of *Herbaspirillum spp.*; B2 – IAC-HT-8 of *Herbaspirillum spp.*; and B3 – IAC-AT-8 of *Azospirillum spp.*), and three rates of N as urea at 0, 60 and 120 kg ha^{-1}, denoted as N0, N60, and N120, respectively. According to Baldani and Baldani (2005), it is important to select the most appropriated endophytic diazotrophic bacterium for each crop.

The application of endophytic bacteria was achieved using 2 g of powdered peaty inoculum per 150 g of seeds. Each gram of inoculum contained a bacteria population of 10^9. Wheat was sown on June 5, 2006 with 80 viable seeds per meter and seedlings were emerged on June 11,

2006. Planting rows were oriented east-west (80° azimuth) and were spaced on 0.2 m. Plots were 5.0 m long and 1.2 m wide. According to the fertilizer recommendations, 1/3 of the total N dosage was applied at planting and the remaining 2/3 was applied as top dressing on July 20, 2006. Based on the soil test, phosphorus (P) and potassium (K) were applied at a rate of 200 kg ha^{-1} using 0-20-20 N-P-K (Raij et al., 1997).

Reflectance factor and LAI

Remote sensing data consisted of conical-directional reflectance factor spectra collected using a FieldSpec Pro FR portable field spectroradiometer (Analytical Spectral Devices Inc., 2006), which operates from 350 to 2500 nm wavelengths of the electromagnetic spectrum. A 25° field of view lens was used, positioned at nadir 0.76 m above the canopy, defining an area of approximately 900 cm^2 above the canopy. The spectral resolution varies from 3 nm (350-700 nm) to 10 nm (700-2500 nm). Radiometric measurements were taken at about 11:00 a.m. using the following protocol: a) signal–to-noise optimization as a function of change in radiance measured on a reference panel (Spectralon); b) reading radiance from the reference panel; and c) reading radiance from the canopy of each plot. The conical-directional reflectance factor (ρ) was obtained from these measurements, which is the ratio between the spectral radiance of the canopy and the spectral radiance of the reference panel, maintained under the same conditions of illumination and target geometry (Milton, 1987). No smoothing procedure was applied after data collection. Due to poor signal-to-noise ratio, noisy data associated with the 1350-1450 nm, 1750-2020 nm and 2350-2500 nm spectral intervals were omitted from the analysis.

Non-destructive LAI measurements were performed early in the morning (more diffuse and less direct solar radiation) with a LAI-2000 Plant Canopy Analyzer (LI-COR Biosciences Inc., 2006). Radiometric as well as LAI measurements were made on three dates: 1) July 21, 2006 when the second node of the wheat plants become visible (jointing; Feekes 7.0); 2) August 4, 2006 when the awns were visible and the heads were emerging through the slit of flag leaf sheath (heading; Feekes 10.1); and 3) August 21, 2006 when grains became milky ripe (ripening; Feekes 11.1), according to the growth stage description for cereals given by Large (1954).

Continuum removal

Continuum removal is a technique proposed by Clark and Roush (1984) that consists of normalizing the absorption features of the reflectance spectrum by using a straight line

(continuum), which delimits the edges of absorption features (Figure 1).

Figure 1

The mathematic formula for continuum removal of the reflectance spectrum is given in Equation 1 (Clark and Roush, 1984):

$$\rho_{CR\lambda} = \frac{\rho_{original\ \lambda}}{\rho_{continuum\ \lambda}} \ , \qquad \text{Eq. 1}$$

where λ is the wavelength; ρ_{CR} is the normalized conical-directional reflectance factor, that is, with the continuum removed; $\rho_{original}$ is the original conical-directional reflectance factor; and $\rho_{continuum}$ is the conical-directional reflectance factor of the continuum line.

The continuum line can be expressed mathematically by a linear equation (Equation 2), where slope (k) is obtained from Equation 3 and where y-intercept (w) is obtained from Equation 4, adjusted to the upper and lower limits (user defined) of each absorption band,

$$\rho_{contínuum\lambda} = w + k\lambda \qquad \text{Eq. 2}$$

$$k = \frac{\rho_{original(M)} - \rho_{original(m)}}{\lambda_M - \lambda_m} \qquad \text{Eq. 3}$$

$$w = \frac{\left[\rho_{original(m)}.(\lambda_M - \lambda_m)\right] + \left[-\lambda_m.(\rho_{original(M)} - \rho_{original(m)})\right]}{\lambda_M - \lambda_m} \ , \qquad \text{Eq. 4}$$

where *M* and *m* represent the upper and lower limits, respectively, of the absorption band under analysis. Other details of continuum removal can be found in Clark and Roush (1984), Kokaly and Clark (1999) and Noomem et al. (2005).

After continuum removal, two spectral parameters (band depth P_B and band area A_B) can be obtained (Figure 1). P_B is calculated from the definition of the position of the reference λ ($\lambda_{reference}$) from which P_B is measured. One way to define $\lambda_{reference}$ consists of locating the λ at which the greatest depth occurs (least ρ_{CR} value). Another way is to adopt a $\lambda_{reference}$ value from the literature. In this study, the lower limit (*m*), the upper limit (*M*) and the $\lambda_{reference}$, for each absorption band, were defined based on Gao (1996), Curran et al. (2001), Schmidt and Skidmore (2003), and Galvão et al. (2005) and are given in Table 1. The absorption band depth (P_B) is the distance between $\rho_{continuum}$ (=1) and ρ_{PCR} at the position of $\lambda_{reference}$ (Equation 5):

$$P_B = 1 - \rho_{CR\lambda} \quad \text{Eq. 5}$$

Table 1

The absorption band area (A_B) is formed by both the $\rho_{continuum}$ line as by the ρ_{CR} band (Figure 1c) and is calculated through numerical approximation (Equation 6), which considers the area of the trapezoid in each interval of λ measured by the sensor between the limits *m* and *M* shown in Table 1.

$$A_B = \sum_{i=m}^{M} \frac{(\rho_i + \rho_{i-1})}{2} . (\lambda_i - \lambda_{i-1}) \ , \quad \text{Eq. 6}$$

where A_B is the absorption band area, and *i* represents the λ interval measured by the sensor along the spectrum between the absorption band limits *m* and *M* (Table 1). It should be emphasized that the λ interval is defined by the spectral resolution of the sensor.

In the present study, the NDVI was calculated using Equation 7, as described by Daughtry et al. (2000):

$$NDVI = \frac{\rho_{801} - \rho_{670}}{\rho_{801} + \rho_{670}} \ , \quad \text{Eq. 7}$$

where ρ_{801} and ρ_{670} represent the conical-directional reflectance factors measured at 801 nm and 670 nm, respectively.

Statistical analysis

In order to evaluate the impact of the different N and endophytic bacterial treatments on the biophysical parameters (LAI and grain yield) and spectral parameters (A_B, P_B and NDVI), the following statistical procedures were used: (a) Bartlett's test to confirm homoskedasticity; (b) analysis of variance (ANOVA) to determine whether treatment means differed from each other; and (c) Tukey's test to identify significant differences detected by ANOVA.

To improve the precision of the correlations between biophysical and spectral parameters without using assumption for sample distribution, the *bootstrapping* technique (Efron, 1982) was used with 1,000 repetitions with replacement (combinations of the 72 plots). The bootstrap statistics consists of the random collection of a predetermined number of samples

from a population for *n* times, yielding *n* correlations, with the objective of reducing error associated with the collection of a non-representative sample. Analyses were performed on data collected during the jointing, heading and ripening growth stages. Results from these estimates were evaluated through histograms, wherein the probability of occurrence of a result is given by the area below the curve.

RESULTS AND DISCUSSION

Effects of treatments on biophysical parameters

A preliminary analysis for variance homogeneity using Bartlett's test revealed that LAI and grain yield were homoskedastic. The ANOVA of the LAI values showed that only the N factor differentiated the mean values (Table 2) while Sala et al. (2007) found that both N and bacteria affected the plant dry matter weight. According to Serrano et al. (2000), LAI was strongly dependent on N application rates. For the jointing and ripening growth stages, the N60 and N120 treatments had a significant effect on LAI values in comparison with treatments with no N input. However, significant difference in LAI between the N60 and N120 treatments was observed only for the heading stage (Table 2). This behavior was probably due to differences among growth stage and the effect of additional supply of N on LAI became evident only at the heading stage as was also observed by Alley et al. (2009). No interaction between bacteria and N for LAI was observed during any of the three analyzed growth stages (Table 2).

As indicated in the last column of Table 2, bacteria, N rates and the interaction between these two factors affected grain yield at statistical significance levels of 1%, 1% and 5%, respectively. The interactive effects of bacteria and N rates on grain yield was observed only for bacterium B2 which significantly increased grain yield in the absence of N (B2N0), as shown in Table 3. In spite of the absence of an interactive effect on LAI, the B2N0 treatment promoted a greater grain yield (2046 kg ha^{-1}). This might be related not only to the greater efficiency of the B2 bacterium in N fixation but also to a greater efficiency in the translocation of carbohydrates from source (leaves) to sink (grain) due to the greater amount of roots produced by B2 bacterium (Sala et al., 2007). Despite the absence of statistical significance, the same comment can be made for treatment B2N60, which produced similar grain yield. These results demonstrates the importance of the correct diazotrophic bacterium selection for each crop (Baldani & Baldani, 2005). In N60 and N120 levels, the bacteria did not significantly affect grain yield, whose effects were probably obliterated by the greater N availability. Thus, B2 was the most adapted bacterium strain to provide N to wheat plants.

Table 2

Table 3

Effects of treatments on spectral parameters (A_B, P_B and NDVI)

The preliminary analysis for variance homogeneity using Bartlett's test also revealed that P_B, A_B and NDVI were homoskedastic. Tables 4 and 5 show results from the analysis of variance applied to A_B and to P_B of the three absorption bands centered at 665 nm (chlorophyll), 982 nm (leaf water) and 1205 nm (leaf water) for the growth stages of jointing (July 21, 2006), heading (August 4, 2006) and ripening (August 21, 2006). Table 6 presents the results for the NDVI.

The bacterial strains had no significant effect on A_B, P_B or NDVI. On the other hand, N rates showed significant effects on these spectral parameters in all three growth stages. At jointing stage, N60 and N120 increased values of spectral parameters significantly (except P_B 665 nm and P_B 982 nm). During initial development, plant growth was positively affected by increased N availability producing more green biomass and LAI (Serrano et al., 2000). Such an increase resulted in larger values of NDVI and in better-defined chlorophyll absorption bands and leaf water spectral features because these features are also sensitive to LAI (Gao, 1996). Consequently, the N levels were distinguished by most of the spectral parameters (A_B, P_B and NDVI). This indicated that more N fertilization promoted the photosynthetic activity (A_B 665 nm and NDVI) and a green biomass and LAI increase (A_B 982 nm, A_B 1205 nm, P_B 1205 nm and NDVI). For heading and ripening stages, only N0 was significantly different from N60 and N120 for all spectral parameters (Tables 4, 5 and 6).

Both A_B and P_B values were smaller during ripening stage (Tables 4 and 5), in which wheat onset its senescence (Mutanga et al., 2003). While P_B separated N0 from both N60 and N120 in the jointing stage, but A_B allowed to differentiate at all N levels. Since A_B is related to the depth and width of each absorption band, results indicated that increased N levels produced deeper and broader absorption features. Thus, the absorption band at a specific wavelength (P_B) was less sensitive to N than the absorption band at a broader spectral interval (A_B).

The P_B and A_B values in the 982 nm and 1205 nm leaf water absorption bands first increased in both jointing and heading stages, and then decreased during the ripening stage as expected (Tables 4 and 5). This behavior was slightly different for the chlorophyll absorption band (665 nm) that presented slightly larger P_B and A_B values in the jointing stage than in the heading. Similar behavior was observed for NDVI (Table 6). This fact might be attributed to the

significant N effect observed during the jointing stage. Both the 665 nm absorption band parameters (Tables 4 and 5) and the NDVI (Table 6) decreased towards the ripening stage as expected. During the ripening stage, the spectral reflectance of wheat canopy tends to increase in the red band due to reduced photosynthetic activity of the leaves, and decrease in the near infrared range due to reduced multiple scattering (Goel, 1988; Mutanga et al., 2003), which reduced the NDVI values. Furthermore, the ripening stage is also characterized by a decrease in the leaf water content sensed by the instrument, which produces shallower absorption bands at 982 and 1205 nm. In general, results of Tables 4, 5 and 6 were in agreement with those reported by Mutanga et al. (2003; 2005).

Table 4

Table 5

Table 6

Correlations between biophysical and spectral parameters

Figure 2 shows the bootstrap correlations for the relationships of LAI with P_B values at 665 nm, 982 nm and 1205 nm and with NDVI at jointing (Figure 2a), heading (Figure 2b) and ripening (Figure 2c) growth stages. Results for the association of LAI with A_B and NDVI values are presented in Figure 3. The P_B is related to the energy absorbed in a specific wavelength, while A_B comprises all the energy absorbed by crops in a broad spectral interval around the feature (Clark and Roush, 1984; Kokaly and Clark, 1999). In Figure 2, correlation values increased from the jointing to the heading stage, and then decreased in the ripening stage. The NDVI and the depth of the 1205 nm leaf water absorption band were strongly correlated (r = +0.80), which presented the largest frequency of high correlations in the heading stage. Similar results between LAI, A_B and NDVI were observed in Figure 3 because of the expected association between P_B and A_B.

Figure 2

Figure 3

Figure 4 presents the distribution of correlation coefficients for the association of yield with P_B values at 665 nm, 982 nm and 1205 nm and with NDVI at jointing (Figure 4a), heading (Figure 4b) and ripening (Figure 4c) growth stages. Results for the association of LAI with A_B and NDVI values are illustrated in Figure 5. Differently from LAI values (Figures 2 and 3), correlations of grain yield with P_B, A_B and NDVI (Figures 4 and 5) were much better (average of 0.72 for NDVI) in the heading than in the jointing stage (average of 0.54 for NDVI). In the heading stage, the range of correlation coefficients was also less variable than in the other two stages. All spectral parameters presented similar correlation results in the heading stage (Figures 4b and 5b). In general, the results for yield were somewhat better than those obtained for LAI in this stage.

The mean correlation values from *bootstrap* analysis are shown in Table 7. In agreement with previous results, higher values of average correlation coefficients were observed in the jointing and heading stages for the relationships of LAI with NDVI and spectral parameters associated with the 1205 nm leaf water absorption band. For grain yield, the spectral parameters under analysis showed similar average correlations in the heading stage. Thus, the heading stage (August 4, 2006) presented the largest correlation coefficients for LAI and grain yield, which is in agreement with results obtained by Mullen et al. (2003) when studying wheat crop treated with different N levels.

Figure 4

Figure 5

Table 7

CONCLUSIONS

The evaluation of the impact of different N treatments and endophytic bacteria on biophysical (yield and LAI) and spectral (NDVI, P_B and A_B) parameters of wheat showed that N was the most important factor. The B2 was the only bacterium to increase wheat grain yield in the absence of N (B2N0 treatment), which might be attributed to greater root/shoot relationship and consequent increase in translocation of carbohydrates from source to sink. However, this was not observed in the presence of N. For LAI, only N rates had a significant effect. Spectral parameters presented increased values from jointing to heading stages in response to increased N levels.

Comparison of the performance of NDVI and absorption band parameters showed that NDVI was the best to estimate biophysical parameters closely followed by the leaf water absorption

band at 1205 nm. High values of correlation coefficients (0.70 to 0.80) for LAI with spectral parameters were observed at both jointing and heading stages. For grain yield, the strong correlations were observed only at heading stage indicating that it is the best timing to acquire remote sensing data to estimate grain yield.

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Table 1. Upper and lower limits of absorption bands and $\lambda_{reference}$ for continuum removal.

	Lower limit (*m*)	Upper limit (*M*)	$\lambda_{reference}$	Associated components
1st. absorption band	550 nm	720 nm	665 nm	Chlorophyll
2nd. absorption band	950 nm	1015 nm	982 nm	Water
3rd. absorption band	1140 nm	1260 nm	1205 nm	Water

Table 2. Analysis of variance for the LAI and grain yield (kg ha^{-1})

Factors	*n*	LAI						Grain yield kg ha^{-1}	
		21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening			
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
B0	18	3.79 NS	1.36	3.55 NS	0.82	3.34 NS	0.79	2949 a	847.7
B1	18	3.79 NS	1.55	3.64 NS	1.05	3.46 NS	0.96	3225 b	984.8
B2	18	4.04 NS	1.47	3.61 NS	0.98	3.67 NS	0.98	3452 b	700.1
B3	18	3.72 NS	1.36	3.57 NS	0.97	3.93 NS	1.19	3237 b	998.9
N0	24	2.45 a	0.75	2.69 a	0.49	2.91 a	0.79	2107 a	428.5
N60	24	4.20 b	1.17	3.70 b	0.61	3.65 b	0.59	3498 b	288.9
N120	24	4.86 b	1.00	4.39 c	0.77	4.24 b	1.08	4041 c	322.3
		F test	*p*-value	F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		0.32	0.81	0.07	0.97	1.79	0.16	9.17	<0.01**
Nitrogen (b)		34.84	<0.01**	38.89	<0.01**	15.91	<0.01**	287.00	<0.01**
Interaction (a X b)		0.30	0.93	0.27	0.95	1.36	0.24	2.50	0.03*

*, ** Significant at the 5% and 1% levels, respectively; NS – not significant: values followed by the same letter do not differ from each other at the 5% probability level.

Table 3. Analysis of the interaction between bacteria and Nitrogen (N) for grain yield (kg ha^{-1}).

Bacteria	N	Grain yield[a]
B0	N0	1832 a
B1	N0	2046 a, b
B2	N0	2568 b
B3	N0	1983 a
B0	N60	3320 c
B1	N60	3455 c
B2	N60	3750 c, d
B3	N60	3467 c
B0	N120	3696 c, d
B1	N120	4173 d
B2	N120	4037 d
B3	N120	4260 d

[a] Means followed by the same letter do not differ significantly at the 5% level.

Table 4. Analysis of variance for area (A_B) values of the absorption bands centered at 665 nm, 982 nm and 1205 nm.

A_B 665 nm

Factors	*n*	21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
N0	24	99.22 a	4.34	86.65 a	8.04	58.08 a	13.12
N60	24	105.04 b	2.64	102.82 b	3.76	71.39 b	9.09
N120	24	108.17 c	1.59	104.94 b	2.26	74.27 b	9.85
Analysis of variance		F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		2.04	0.12	0.30	0.83	0.13	0.94
Nitrogen (b)		24.86	<0.01**	23.15	<0.01**	6.82	<0.01**
Interaction (a X b)		0.35	0.91	1.35	0.25	0.33	0.92

A_B 982 nm

Factors	*n*	21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
N0	24	1.50 a	0.45	1.63 a	0.71	1.44 a	0.7
N60	24	1.92 b	0.41	2.36 b	0.47	2.05 b	0.52
N120	24	2.27 c	0.24	2.62 b	0.31	2.03 b	0.63
Analysis of variance		F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		0.50	0.68	0.12	0.95	0.08	0.97
Nitrogen (b)		34.51	<0.01**	46.01	<0.01**	12.00	<0.01**
Interaction (a X b)		0.38	0.89	1.83	0.11	0.39	0.88

A_B 1205 nm

Factors	*n*	21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
N0	24	6.27 a	0.77	6.38 a	1.09	5.44 a	1.7
N60	24	7.08 b	0.51	8.21 b	0.87	7.08 b	1.2
N120	24	7.84 c	0.58	8.81 b	0.81	7.37 b	1.25
Analysis of variance		F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		0.51	0.68	0.28	0.84	0.20	0.90
Nitrogen (b)		47.73	<0.01**	79.51	<0.01**	13.93	<0.01**
Interaction (a X b)		0.19	0.98	0.51	0.80	0.40	0.88

*, ** Significant at the 5% and 1% levels, respectively; NS = not significant; values followed by the same letter do not differ from each other at the 5% probability level.

Table 5. Analysis of variance for depth (P_B) values of the absorption bands centered at 665 nm, 982 nm and 1205 nm.

		P_B 665 nm					
Factors	*n*	21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
N0	24	0.877 a	0.0193	0.796 a	0.0449	0.559 a	0.0948
N60	24	0.897 b	0.0107	0.868 b	0.0148	0.654 b	0.0628
N120	24	0.901 b	0.0101	0.864 b	0.0087	0.672 b	0.0695
Analysis of variance		F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		2.07	0.11	0.23	0.88	0.39	0.76
Nitrogen (b)		20.32	<0.01**	23.05	<0.01**	4.33	0.02*
Interaction (a X b)		0.51	0.8	1.02	0.42	0.44	0.85
		P_B 982 nm					
Factors	n	21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
N0	24	0.039 a	0.0090	0.043 a	0.0143	0.039 a	0.0157
N60	24	0.047 b	0.0097	0.058 b	0.0086	0.051 b	0.0138
N120	24	0.054 b	0.0064	0.063 b	0.0068	0.050 b	0.0153
Analysis of variance		F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		0.74	0.53	0.14	0.94	0.07	0.98
Nitrogen (b)		38.18	<0.01**	48.6	<0.01**	11.69	<0.01**
Interaction (a X b)		0.43	0.86	1.86	0.1	0.36	0.9
		P_B 1205 nm					
Factors	*n*	21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
N0	24	0.080 a	0.0091	0.082 a	0.0130	0.070 a	0.0210
N60	24	0.091 b	0.0064	0.105 b	0.0102	0.090 b	0.0149
N120	24	0.100 c	0.0070	0.111 b	0.0099	0.093 b	0.0156
Analysis of variance		F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		0.5	0.66	0.45	0.72	0.2	0.9
Nitrogen (b)		18.4	<0.01**	46.59	<0.01**	13.69	<0.01**
Interaction (a X b)		0.2	0.99	0.3	0.93	0.35	0.91

*, ** Significant at the 5% and 1% levels, respectively; NS = not significant; values followed by the same letter do not differ from each other at the 5% probability level.

Table 6. Analysis of variance for NDVI values.

Factors	*n*	NDVI					
		21/07/2006 jointing		04/08/2006 heading		21/08/2006 ripening	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
N0	24	0.89 a	0.02	0.82a	0.05	0.65 a	0.11
N60	24	0.92 b	0.01	0.92 b	0.02	0.76 b	0.08
N120	24	0.94 c	0.01	0.93 b	0.01	0.79 b	0.07
Analysis of variance		F test	*p*-value	F test	*p*-value	F test	*p*-value
Bacteria (a)		0.6	0.6	0.24	0.87	0.15	0.93
Nitrogen (b)		43.5	<0.01**	69.06	<0.01**	15.21	<0.01**
Interaction (a X b)		0.3	0.95	0.37	0.9	0.49	0.82

*, ** Significant at the 5% and 1% levels, respectively; NS = not significant; values followed by the same letter do not differ from each other at the 5% probability level.

Table 7. Mean correlation coefficients from *bootstrap* analysis for the relationships of grain yield and LAI with absorption band depth (P_B), area (A_B) and NDVI values.

Spectral Parameter	21/07/2006 jointing	04/08/2006 heading	21/08/2006 ripening
		LAI	
P_B 665 nm	0.58	0.70	0.58
A_B 665 nm	0.76	0.82	0.58
P_B 982 nm	0.52	0.66	0.37
A_B 982 nm	0.57	0.69	0.41
P_B 1205 nm	0.73	0.80	0.54
A_B 1205 nm	0.72	0.80	0.55
NDVI	0.75	0.81	0.58
		Yield	
P_B 665 nm	0.54	0.72	0.53
A_B 665 nm	0.54	0.71	0.54
P_B 982 nm	0.38	0.70	0.43
A_B 982 nm	0.46	0.70	0.46
P_B 1205 nm	0.49	0.74	0.51
A_B 1205 nm	0.50	0.73	0.52
NDVI	0.54	0.72	0.53

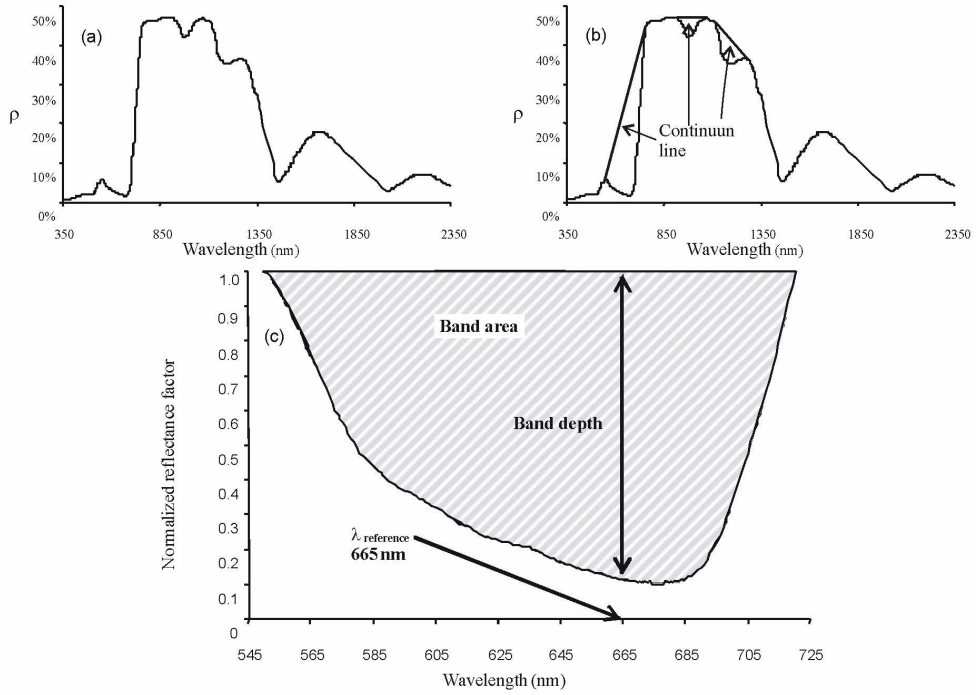


Figure 1 – Schematic representation of the continuum removal. a) Reflectance spectrum of green vegetation; b) Continuum lines delimiting the edges of the three studied absorption bands (chlorophyll at 665 nm; leaf water at 980 nm and 1205 nm); and c) Normalized reflectance spectrum after continuum removal to isolate the absorption bands and to allow subsequent calculation of their depth and area. Source: Adapted from Pu et al. (2003).

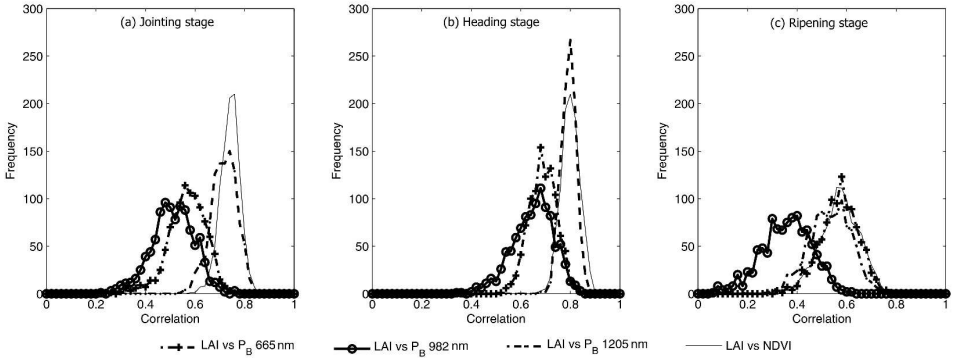
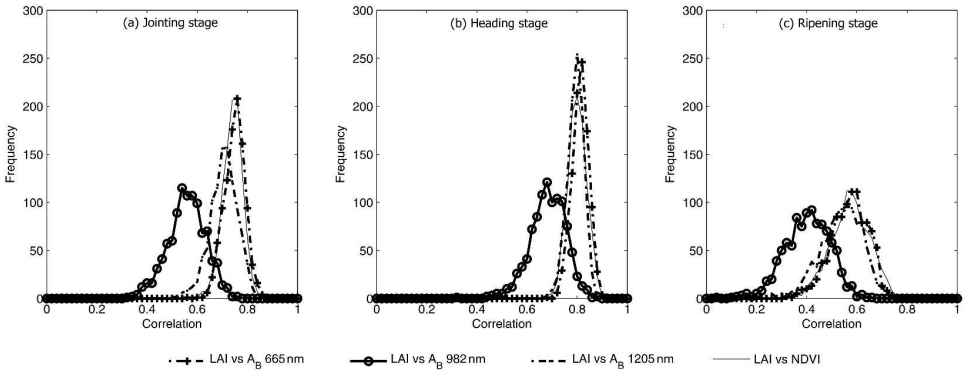


Figure 2 – Frequency of the correlation coefficients from bootstrap analysis for the relationships of LAI with band depth (PB) values of the 665 nm, 982 nm and 1205 nm absorption bands and with NDVI for the: (a) jointing; (b) heading; and (c) ripening wheat growth stages.



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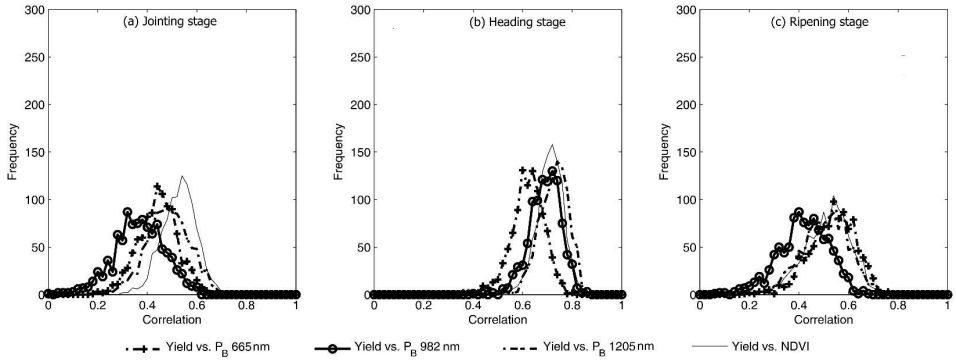

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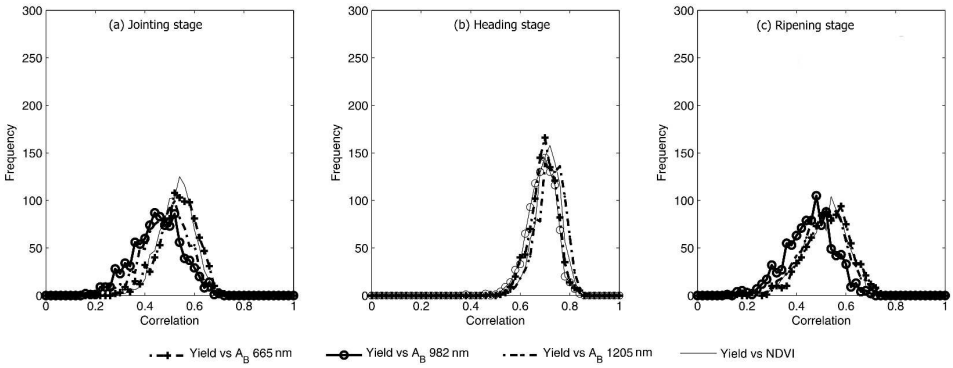

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