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Full Research Article

Determining Wavelenth for Nitrogen and Phosphorus Nutrients Through Hyperspectral Remote Sensing in Wheat (*Triticum aestivum* L.) Plant

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Abstract

For precision management of nitrogen (N) and phosphorus (P) nutrients, soil or plant test techniques are either expensive or time taking. Hyperspectral remote sensing plays an important role for minimizing loss and judicious use of supply nutrient in timely fashion. To determine wavelength from hyperspectral data for N and P nutrient, glasshouse experiments were conducted at Dookie, Australia in year 2006 and 2007 on wheat (*Triticum aestivum* L. cv. Ruby) with nutrients (Control, 10% N, zero N, 10% P and Zero P) and harvest dates (2 leaf, Z12; Initiation of Tillering, Z20; Stem Elongation, Z30; booting, Z40 and grain filling, Z70). Results showed that the control N and P plants can be separated from stressed plant at all investigated growth stages and in a specific wavelength range. Control N and stressed plants are found significantly separable at 212–385 nm and 440–443 nm at Z12, while at Z20, separable in blue, green, red, red edge and NIR; at Z30, separable at 244–521.5 nm and 591–1100 nm and at Z40, separable at 350–415 nm and 706–934.5 nm; at Z70, separable at 355–515.5 nm, 617–695 nm and 726–1075 nm. Likewise, results showed that control P and stressed plant were found significantly separable at 245.5–504 nm and 549–1100 nm at Z12, while at Z20, separable at entire wavelength ranges except at 490–512 nm, 638–69 nm and 732–762 nm. At Z30, significantly separable P stressed were found at 227.5–230 nm and 364–367 nm; at Z40, 544.5–612.5 nm and 687–761.5 nm and at Z70, 355–521 nm, 644–692 nm and 716–929.5 nm. The shifting of the wavelength among the different plant growth stages was also found.

1. Introduction

Soil and plant analyses are the main techniques for the estimation of N and P nutrients. These tests are costly, time consuming and do not represent the whole field because of the variability of N and P levels in soil and other factors like moisture, climatic and plant conditions. Because of the large soil variability, traditional testing either becomes unrepresentative or costly because of many samples that need to be collected across fields. With increase in the use of variable rate technology and precision agriculture, multi-spectral/hyperspectral remote sensing has the capability to identify and estimate remotely the nutritional status of plants. The hyperspectral remotely sensed estimate provides accurate, timely and fast spatial and temporal measurements of plant N and P (Mahajan et al., 2014). The remote sensing collects reflectance data from the leaf surface of plant or crop canopy in the different wavelength range especially in visible and NIR (Ozyigit and Bilgen, 2013). These spectral studies provide an estimate of the sensitive wavelengths responsible for the determination of N and P in crops or plants. From these wavelengths, sensitive vegetation indices could be derive for a better estimation of deficiency of plant N (Raper et al., 2013) and P in advance of actual deficiency visibly shown by crops or plants. This could lead to the prompt application of N and P to remedy deficiency and to reduce the risk of excessive application either from fertilizer or manures. Thus, for identifying N and P levels in plants, hyperspectral studies are required to determine the appropriate wavelengths.

As N and P are essential mineral elements and most limiting nutrients for plant growth, adequate availability of these nutrients is essential for profitable grain, forage and animal production. On the one hand, deficiencies in these nutrients are detrimental for the growth and yield of plants and on the other hand excessive application of fertilizer or manures leads to nitrification or eutrophication. N and P stresses have been monitored and estimated through specific electro-magnetic

wavelengths (Curran et al., 2001; Fonteneli et al., 2004; Osborne et al., 2004; Petisco et al., 2005; Mobasheri and Rahimzadegan, 2012). It is found that these stresses have been detected in plants using the visible and NIR spectrums i.e., in blue, green, red and infra red regions (Sembiring et al., 1998; Osborne et al., 2002; Zhao et al., 2003; Osborne et al., 2004; Ayala-Silva and Beyl, 2005; Bogrekci and Lee, 2005; Mistele et al., 2010). It is important that the estimation of nutrients is better using the plants than soil, because plants are seen easily than soil (Schepers et al., 1996; Sembiring et al., 1998; Asner et al., 1999).

Research has been focused to determine the appropriate wavelengths to characterize N nutrient deficiency in plants. The visible and NIR regions of electromagnetic spectra are useful to characterize the N stress of plants (Thomas and Oerther, 1972; Blackmer et al., 1994; Tarpley et al., 2000; Graeff and Claupein, 2003; Fridgen and Varco, 2004) in different crops. To better estimate nutrients from reflectance studies, vegetation indices (VIs) have been derived (Ma et al., 1996; Sembiring et al., 2000; Hansen and Schjoerring, 2003; Zhao et al., 2005; Ansari et al., 2006; Zhu et al., 2007; Li et al., 2008; Zhu et al., 2008). However, the estimation of N in wheat flag leaf is also possible by using whole spectra in the region of visible and NIR (Bonfil et al., 2005). The spectral reflectance is changed depending on the growth stage or phenologies of plants. Thus, there is a need to know the spectral wavelength or vegetation indices at different growth stages. Most of the research shows that they use either whole crop growth or some vegetative growth stages for the derivation of vegetation indices. For example, N has been predicted at the silking stage in corn (Schepers et al., 1996) and at the flowering stage in cotton (Fridgen and Varco, 2004). Studies show that the spectral signature of N deficient plants changed with the plant growth stages (Osborne et al., 2002; Ayala-Silva and Beyl, 2005; Ansari et al., 2008). The application of N in the also changed the position of wavelength from longer to shorter or vice versa in the visible and NIR ranges (Fridgen and Varco, 2004; Li et al., 2006; Mutanga and Skidmore, 2007), which shows that the different concentrations of the plant N exhibit differently for detecting N (Mistele et al., 2010). Hence, the consideration of individual growth stage is important for deriving the vegetation indices.

P deficiency has also been found by the plants reflectance in the visible to NIR regions. Similar to N, the spectral reflectance of P stressed plant is changed depending on the growth stage or phenologies of plants. NIR reflectance spectroscopy analysis of phosphorus is carried out in sugarcane leaves (Chen et al., 2002) and other crop including wheat (Sembiring et al., 1998; Ayala-Silva and Beyl, 2005; Jorgensen et al., 2006). Thus, there is a need to know the spectral wavelength or vegetation indices at different growth stages. Most of the research shows that they use either whole crop growth or some vegetative growth stages for the derivation of vegetation indices. In regards to exact wavelength for N and or P estimation by the leaf spectral properties, there is discrepancy among the authors. Thus, this chapter investigates the determination of spectral wavelengths which represent N and P deficiencies in the plant at different growth stages.

Thus, the glasshouse experimentation of wheat in pots is carried out in order to achieve the objective of determining best wavelength, so that vegetation index for N or P estimation can be carried out. This paper described about the N and P experimentation for the selection of wavelengths to separate N or P stressed plants from the control plants.

2. Materials and Methods

2.1. Experimentation for N and P nutrients with harvesting dates

The experiments were conducted at a glasshouse in year 2006 and 2007 at Dookie College, Melbourne School of Land and Environment (formerly Faculty of Land and Food Resources), the University of Melbourne, Victoria (Australia). Dookie College is situated at a latitude of 36°37′ S and longitude of 145°70′ E and elevation of 180 m amsl.

The first experiment was sown in March 8, 2006 and second experiment in August 30, 2007. These were harvested in August 3, 2006 and December 1, 2007, respectively. The experiments were laid in completely randomized blocks, and each treatment was replicated seven times for the first experiment and eight replications for the second experiment. Temperatures in the glasshouse were maintained by cooling on hot days and nights. It's ranged from 21.8±3 °C during the day to 12.8±2 °C at night in winter and 30.2±4 °C during the day to 25.6±3 °C at night±3 °C in summer.

Bread wheat (*Triticum aestivum* L. cv Ruby) was grown in the black plastic pots (25×30 cm^2) filled with vermiculite (grade 2) as growing media. Vermiculite is used as a growth medium; as this does not adsorb or release P (data is not presented here). The two harvest dates were at booting (Zadoks scale 40, Z40) and at grain filling (GF, Z70) for experiment in the year 2006. In the year 2007 experiment, plants were harvested at 2 leaf (Z12), Initiation of Tillering (IT, Z20) and Stem Elongation (SE, Z30) growth stages. At Z12, Z20, Z30, Z40 and Z70, the crop is harvested at 14, 27, 54, 84 and 145 DAS (days after sowing).

The nutrient treatments were (a) Control (full nutrient, 100% N and P), (b) 10% N, (c) zero N, (d) 10% P and (e) Zero P. Control treatment was supplied with appropriately modified Hoagland's

solution described in Table 1a and 1b. For creating other four treatments, an extra nutrients and stock solutions was needed i.e., stock solutions 'G to J' (Table 1a). Stock solutions were stored in dark glass bottles to avoid disintegration of nutrient solutions. The five treatments were made by selecting required stock solutions from Table 1a. From these stock solutions, 5 ml of each required stock solution for a treatment was taken to dissolve in 10 liters of distilled water in a 20 liter plastic container. All stock solutions become diluted 2000 times and the actual concentration of applied nutrient are presented in Table 1a. The pH was adjusted to 6.5 after dissolving of 1.0 N NaOH solutions to make sure that the nutrients become available to the plants Cultural Operation.

The selected bold seeds were chosen (the average weight was 40–45 g 100 seed^{-1}) and were kept in metal tray over the soaking paper. Deionised water was sprinkled over the covered seeds and they were kept in a cool place, so that the paper remained moist during germination. The deionised water was applied daily over the paper. In 3–4 days, seedling with well developed roots (average 2 cm root length and 0.5 cotyledon length) were transferred to the nutrient soaked pots. Each pot was filled with 1 kg of fine vermiculite (grade 2). The vermiculite in each pot was saturated with 1000 ml of nutrient solutions (Table 1a and 1b). The nutrient solution was sprinkled using a perforated cap from a 3 liter plastic bottle. The pots were left to drain and on the second day, eight pre-germinated seeds of wheat were placed in each pot at 2.0 cm deep. After emergence of the wheat seedlings (four days from sowing), each pot was soaked with 750 ml of secondary nutrient solution (Table 1a and 1b) as the treatment^{-1}. After seven days of seedlings sowing, the nutrient solutions were applied thrice a week to saturate the pot in the growing period on both experiments until one day before each harvest. More frequent nutrient solution was applied in hot weather. The plants were thinned to five pot^{-1} at 14 DAS (days after sowing) i.e., at 2 leaf stage (Z12), keeping the most uniform plants.

The plants from 70 pots (5 nutrient treatments×2 harvest dates×7 replications) for experiment 2006 and 80 pots (5 treatments×2 harvest dates×8 replications) for experiment 2007, were uprooted at each of the harvest dates. After each harvest, 3 plants from each pot were put in air tight plastic bags to record spectral data. After taking spectral data, plant samples were kept in shade to reduce the moisture and then in oven at 60 °C for 48 hours for complete drying for dry matter and P concentration data of the plant. Another 2 plants from each pot were also kept in air tight bags to measure leaf length, leaf width and plant height (growth parameter is not discussed in this paper). All bags were transported in a thermocol container to the laboratory. These samples were kept in a fridge at 4 °C.

2.2. Determining hyper-spectral reflectance data from spectrometer

The Leaf hyper-spectral data was recorded by the EPP2000-UVN-SR (Ultraviolet Visible Near infrared–Spectroradiometer) of Steller Net Inc. which covers 200–1100 nanometer (nm) wavelength range at 0.5 nm interval in the dark and cold room at 18 °C in the laboratory and easily performs the spectroscopy measurements with the Spectra Wiz software which used to accurately measure transmission or reflectance.

2.3. Setting and procedures

Before measuring the percent spectral reflectance, spectra setting were adjusted. The spectral data was smoothed with a five point average calculated as the mean of the reflectance value in the program. The spectra were also adjusted for smoothing to pixel smoothing (Sm), Savitsky Golay (Sg) and display persistence level for avoiding noise, particularly in the infrared and ultraviolet region of the spectrum. The other parameters were also set before the data taking for the

Table 1a: The Stock solutions and their chemical concentrations

Stock	Stock solution concentration (M or ppm)	Nutrient solution concentration (mM or ppm)	Chemical formula
A	2.0 M	1 mM	$Ca(NO_3)_2.4H_2O$
B	0.5 M	0.25 mM	$MgSO_4.7H_2O$
C	1.0 M	0.5 mM	KH_2PO_4
D	558 ppm	0.279 ppm	Fe-EDTA
E	6.6 mM	3.3 µM	H_3BO_3
	1330 ppm	0.665 ppm	$MnCl_2.4H_2O$
	200 ppm	0.1 ppm	$ZnSO_4.7H_2O$
	100 ppm	0.05 ppm	$CuCl_2.2H_2O$
	40 ppm	0.02 ppm	H_2MoO_4
G	2.0 M	1.00 mM	$CaCl_2.2H_2O$
H	0.2 M	0.1 mM	$Ca(NO_3)_2.4H_2O$
I	1.0 M	0.5 mM	KCl
J	0.1 M	0.05 mM	KH_2PO_4

Table 1b: Details of treatment solutions made by respective stock solutions

Treatment	Stock solution								
	A	B	C	D	E	G	H	I	J
1. Control (Full nutrient)	□	□	□	□	□				
2. 10% nitrogen		□	□	□	□	□	□		
3. Zero nitrogen		□	□	□	□	□			
4. 10% phosphorus	□	□		□	□			□	□
5. Zero phosphorus	□	□		□	□			□	

requirements of the instrument. The detached plant leaf was set aside horizontally on the black surface. The viewing angle of sensor for measuring hyperspectral data from leaf surface was adjusted at 45° by the compass. The distance was adjusted at 0.5 cm between sensor tip and leaf to ensure maximum light was captured by sensor after reflecting from the leaf surface and to minimize the scattered diffused^{-1} light from the leaf surface. The light source was away at 30 cm from the leaf surface. The reflectance readings were taken from the first fully opened uppermost leaf. The measurement was taken at the middle portion of the leaf. For the hyperspectral spectral reading, the middle portion of leaves is more important than the other two portions which might be because of the concentration of pigment and P change between the two ends of the leaf at different growth stages (data is not presented here). The percent reflectance of 3 plants pot^{-1} is averaged for one reading replication^{-1}. In the instrumental adjustment, the five scans were set for each sample reading.

Before taking a reading of percent reflectance from the spectrometer, the maximum light and dark reference readings were measured and saved by placing a white board and black foam in front of the sensor to ensure 100% and zero percent reflectance, respectively. In the spectra setting for getting 100% reflectance, the frequency was matched with more than 90% peak in an oval curve from the light in $BaSO_4$ coated white surface (50, reflectance standard 50 mm diameter) to set the white reference and it was saved.

The first reading from the white board was performed with more than 90% saturation light and showed more than 3600 counts in scope mode. This was done to ensure that maximum light was coming to the sensor. The detector integration time was adjusted to increase or decrease the incoming radiation to get the maximum number of radiation counts. That gave 100% reflectance from the white board in the visible and near infrared region (400–1100 nm) but there was no constant reading and fluctuated from 100% in UV region of electromagnetic.

After saving the white board reading from the transmittance mode which was nearly 100% transmittance, a dark reading was performed by placing black foam (a less shiny surface). The dark reading was saved and it gave a zero reading in transmittance mode. The white and dark readings were repeated after each treatment reflectance data. After setting of white and dark readings, the percent reflectances of leaves were recorded in transmittance mode.

The black surface's reflectance was calibrated at zero percent, so that the scattering and or diffused light from the black surface did not affect reflectance. This reflectance was achieved by taking a dark light reflectance. The calibration was made in the artificial and constant illuminated light from a white bulb and UV lamp. UV lamp could be attached with the sensor which was supplied by Steller Net Inc. The percent reflectance of white disc is near 100% in the visible and NIR range, but the percent reflectance was not achieved 100% in the UV region of spectrum and it was greater than 100%. Likewise, the percent reflectance of black surface is near zero, but percent reflectance is greater than zero in UV range. The standard deviation of leaf samples is very less in both reflectance of white and black surface, except in UV range of spectrum.

2.4. Hyperspectral measurements

Hyperspectral reflectance was completed on the day of harvest. Only one treatment samples at a time were taken from fridge and kept in thermocol to avoid moisture loss for sensing reflectance data. In 2006, the hyperspectral reading was started when the crop was in the Z40 stage, while in year 2007, the hyperspectral reading were taking from at the Z12 to at the start of Z30. This was due to the adjustment in setting of the instrument for smoothing such as Sm (pixel smoothing) and Sg (Savitsky Golay) which was zero, while in the year 2007, reflectance was measured from 200−1100 nm with low noise and the value of Sm and Sg was 4 for both.

2.5. Total P analysis of plant sample on dry biomass basis

The dried plant samples were crushed into a fine powder and subjected to ashing in an oven at 500 °C. As the quantity of plant sample was low, a varied amount of plant samples were used for ashing ranging from 0.01 to 2.00 gm. When the different quantity of same sample was tested for the ashing and determining the total plant P then there was no difference in P measurement. After ashing of plant samples overnight and cooling, the samples were digested in 2 ml of 5 M HCl in a water bath at 100 °C for 10 minutes. After adding 5 ml more of 5 M HCl, the volume was made 50 ml. The plant samples were filtered in high quality ashless Whatman filter paper (A43) so that a clear extract was obtained. The extract solutions from the filtrate were used for the colorimetric determination of total plant P (µg P g^{-1} plant dry weight) as described by John (1970). The blue color was developed and absorbance readings were measured at 882 nm in the spectrometer. The standard P solution reading was also calculated by the same procedures. The preparation of the standard was done by using 5.0 mg P L^{-1} secondary standard solution, the 5 M HCl and a mixed color reagent. The total plant P was calculated using the following formula:

$$\text{Total plant P (µg P g}^{-1}) = \frac{[\text{Sample (µg P ml}^{-1}) - \text{blank (µg P ml}^{-1})]}{\text{Weight of Sample (gm)}} \times \text{Dilution}$$

Later, these P concentrations were converted to gm P kg^{-1} dry matter using the total dry matter accumulation of a single plant.

The two way analysis of variation (ANOVA) was performed by the Genstat (11th edition) software to determine the mean comparison. The standard deviation (SD) was derived to differentiate N and P deficient treatments with the control treatment. The coefficient of variations (CV) and standard error of means (SEm±) were also calculated to show the variation in the treatments. SD, CV and SEm± were computed in the excel worksheets. These formulas were:

$SD = \sqrt{[1/N \sum Xi - \overline{X})^2]}$

% CV = $(SD/\overline{X}) \times 100$ *and* $SEM = SD/\sqrt{N}$

Where, Xi=random variable or sample, $\overline{X}$=mean of sample and N=number of observations or sample size

3. Results and Discussion

3.1. Total dry matter and P analysis of a plant

The dry matter accumulation and total plant P concentration were significantly differing from each N and P treatment at all growth stage except at two leaf (Z12) and IT (Z20) growth stage. At early growth stage i.e., Z12 and Z20 growth stages, it was necessary to estimate available N or plant total P concentration from remote sensing technique, because the plant cannot be significantly differ in terms of dry matter or/ and total P at these growth stages. Researchers studied using of reflectance for the early detection of N and P deficiencies in *Zea Mays* L. (Graeff et al., 2001), in spring barley (Christensen et al., 2004) and in rangeland plants (Ozyigit and Bilgen, 2013) and they found that at early growth stage, the N and P nutrient stress could be identified.

3.2. Spectral characteristics in different growth stages

Trends showed that there were absorption bands at UV, blue and red regions more than the green region. There was higher reflectance at the NIR than the visible regions. The SD values were shown in graphs for zero, 10% and control nutrients in the selected wavelengths to show the significant difference among treatments.

The separable wavelength ranges for N stressed were dependent on the growth stages at two leaf to grain filling growth stage in UV to NIR regions of winter wheat plants. At Z12, a separation between 100% N levels and low N levels (0–10% N) plants were found significantly separable at ultraviolet (UV) (212–385 nm) and blue (440–443 nm) wavelength range while at blue, green, red, red edge and the NIR in selected wavelengths were separable at Z20. At Z30, N stressed plants were significantly separable at UV to green (244–521.5 nm) and green to NIR (591–1100 nm) At Z40, N stressed plants were separable at UV to blue (350–415 nm) and red edge to NIR (706–934.5 nm). The percent reflectance's of N stress at Z70 stage was compared with control treatment and found that N stressed plants were separable at UV to green (355−515.5 nm), yellow to red (617−695 nm) and red edge to NIR (726–1075 nm). The studies also showed similarity in identifying the wavelengths for N stress or deficiency (Mistele et al., 2010). Researchers found that N deficiency was detected in the green, red edge and NIR region of spectra at different growth stages (Blackmer et al., 1994; Zhao et al., 2003; Osborne et al., 2002). However, there were deviations of exact wavelength but they lied in the same wave range. Studies showed that with the advancement of crop growth, either wavelength ranges were shifted to higher or lower spectral ranges. Blackmer et al. (1994) studied the reflectance of ear leaves in the visible range and found the green (550 nm) region was best to detect N deficiency in corn. While Zhao et al. 2003 found stressed N plants could be distinguished at the green (552 nm) and red edge (710 nm) in corn. Osborne et al. (2002) studied the canopy hyper spectral reflectance between 350−1000 nm in corn and identified N deficiency at green and NIR region of spectrum with particular wavelengths in each growth stage.

The separable wavelength ranged for P stressed were also dependent on the growth stages. P stress could be identified by hyper spectral reflectance in the UV to NIR regions of spectrum at different growth stages of wheat plants. Similar results were found by other authors and their derived wavelengths were in the range of investigated hyper spectral ranges. At Z12, P stressed plants were found significantly separable at 245.5–504 nm (UV to blue) and 549–1100 nm (green to NIR) , while at Z20 separable at UV (245.5–250.5 nm and 362.5–371 nm), blue (435–489 nm), green to red (513–637.5 nm), red edge (692–731 nm), NIR (763–917 nm, 1082–1094 nm) At Z30, P stressed plants were found significantly separable at 227.5–230 nm and 364–367 nm in UV, while at Z40 separable at 544.5–612.5 nm (green to yellow) and 687–761.5 (red to red edge). Similarly, At Z70, P stressed plants were found separable at the 355–521 nm (UV to green) and 644–692 nm (yellow to red) and 716–929.5 nm (red edge to NIR) (Table 2a and b). The phosphorus concentration of pine needle (*Pinus elliottii*) leaves was found to correlate with the five wavelengths at 584, 612, 1040, 1448 and 1894 nm selected by step wise regression for all three sets of methodology data used i.e., first derivative spectra, band-normalized to centre and band-normalized to area, respectively (Curran et al., 2001). These wavelengths were derived for the early growth stages of tree plants and tested after at the later growth stage of plants. In the other studies above, they mentioned a range of wavelengths which could be used to derive the indices. Likewise at all growth stages there were specific wavelength range. The results also showed that plant deficiencies in N and P could be detected

Table 2a: Separable wavelengths at Z12, Z20, Z30, Z40 and Z70 for differentiating from control to N deficient

Growth stage	Treatment	Ultra violet	Blue	Green	Yellow	Red	Red edge	NIR
				Wavelength (nm)				
Z12	N	212−385	440−443					
Z20	N		402−428	506−664			692−711	721−1100
Z30	N	244−521.5			591−1100			
Z40	N	335−415					706−934.5	
Z70	N		355−515.5			617−695	726−1075	

Table 2b: Separable wavelengths at Z12, Z20, Z30, Z40 and Z70 for differentiating from control to P deficient

Growth stage	Treatment	Ultra violet	Blue	Green	Yellow	Red	Red edge	NIR
				Wavelength (nm)				
Z12	P	245.5−504	549−1100					
Z20	P	245.5−250.5, 362.5−371	435−489		513−637.5		692−731,763−917,1082−1094	
Z30	P	227.5−230, 364−367						
Z40	P				544.5−612.5		687−761.5	
Z70	P		355−521			644−692	716−929.5	

in the early growth stages (Z12 and Z20) prior to visible deficiency symptoms being detected by the human eye (Ansari et al., 2008, Christensen et al., 2004; Osborne et al., 2002; Graeff et al., 2001). The reflectance wavelengths were used to predict P concentration in the NIR (730 and 930 nm) and blue (440 and 445 nm) region of the spectrum in a corn canopy in the early growth stages at V6 and V8 (Osborne et al., 2002). Thus, above finding also suggested that hyper spectral wavelength range in UV, green, red, red edge, NIR could be possible to establish P stress for a specific growth stage and specially at early stages. The identification of nutrient deficiencies in early growth stages will allow remedial treatment without yield penalties especially for P.

3.3. Differentiation between hyperspectral reflectance and nutrient stress levels

The changing of the treatment order in regards to percent reflectance might be due to interaction/ratio of N and P or other physiological reasons. The percent reflectance's of a treatment was changing quantitatively in respect to the other treatment with growth stages at the same wavelength. So, it could be possible that the concentration/availability of N and P are changing at the growth stages. For both N and P, there were significant differences between the deficient treatments (Zero and 10%) and the full nutrient treatment at all growth stages, yet there were no visible deficiency symptoms apparent in Z12 and this finding matched with the work done by Graeff et al., 200; Christensen et al., 2004. The wavelengths that significantly separated the deficient N and P treatments from the control treatment were found in the UV, Blue, Green, Red, Red Edge and NIR spectrum. The Z20 had more separable wavelengths than the other growth stages. Each growth stage had specific bands that separated full nutrient treatments from the deficient treatments. This finding also reported by (embiring et al., 1998; Osborne et al., 2002; Zhao et al., 2003; Ayala-Silva and Beyl, 2005; Bogrekci and Lee, 2005).

In the 2007 data, the three early growth stages were presented, as the initial growth stages were important for the study to rectify the deficiency, especially for P nutrient. In later growth stages, the booting or grain filling stage was also important for applying the nutrient to rectify the nutrient deficiency at least for N. Results showed that in every growth stage, some wave ranges were identified, that were important wavelengths in different growth stages for remedies of nutrients stresses. There were different peaks or range of wavelength values at the same wave range for the different level of N and P stressed plants. That suggested that in a given wave range of spectrum, N and P nutrient stressed plants had different chemical constituents in the leaves which reflect or absorb the particular wavelength (Ansari et al., 2009). This states that with the advancement of growth stage, absorption features of spectrum are changed. This might be due to the change in the pigment concentration in the visible region and the size and number of cells caused in the NIR region. The variation in the reflectance signature of treatments between light regions within a growth stage was most likely due to the different types of plant structures. This was due to the changes in reflectance in the visible spectrum likely being caused by chlorophyll and other pigments where changed in the NIR region was caused by the structure size^{-1} of cell (Ansari et al., 2009). Further studies on the specific structural changed due to the different nutrient levels at the

specific growth stages were warranted in order to target the correct wavelengths.

3.4. Horizontal shift of reflectance

The horizontal shift of reflectance at varying growth stage was depicted in Table 3. At the Z12, the reflectance values were higher for zero N than the control treatment in the range of 200–525.5 626–698.5 716.5−1100. In 526–625.5 nm and 699–716 nm wavelengths range zero N had a lower percent reflectance than the control treatment. The percent reflectance of zero P, 10% P and 10% N treatments were lower than the control treatment across the whole range of the spectrum from 200–1100 nm. At the Z20, the reflectance values were higher than the control treatment in the range of 200–452 and 709.5–1100 nm for zero N, 200–1100 nm for zero P, 200–441 and 717–1100 nm for 10% N and 200–427 nm, 505–652 nm, 687.5–742.5 nm for 10% P. Likewise, in the rest of the wavelengths the percent reflectance were lower than the control treatment. At the Z30, the reflectance values of 10% N were lower than the control treatment across the range of the spectrum. Other treatments had a higher and lower percent reflectance than the control treatment. At the Z40, the reflectance values were higher for the control treatment in the range of 706–934.5 nm for N and 544–612.5, 687–700.5, 701–761.5, 400–700 nm for P. The control treatment had the least reflectance in the 335−415 nm wavelengths range and 10% N treatment had the highest reflectance values. Likewise, at the Z70 growth stage, the control treatment had the highest reflectance and the zero treatments had the least reflectance in 355−515.5 and 726−1075 nm for N and 355−521, 644−692 and 716−929.5 nm wavelengths for P treatments.

3.5. Vertical shift in reflectance

The percent reflectance was ranked from 1−5 based on the lowest to highest percent reflectance of treatment at each growth stage and at each waveband. The rank of the highest percent reflectance was kept 5 and lowest is kept 1. The relative order of a percent reflectance of treatments was inconsistent throughout the plants growth stages of Z12, Z20, Z30, Z40 and Z70. For example, the percent reflectance of Zero P in the blue spectrum was highest at Z12 but lowest at Z20. Similarly, other treatments also showed an inconsistent trend of relative order. Further, zero N, zero P, 10% N, 10% P and control treatments are interchangeable in the wavelength ranges of blue, green, red, red edge and NIR at the different growth stages.

Shift of percent reflectance in vertical axis of N and P stressed plant from the control treatment at different growth stages were depicted in Table 4. There was no shift of wavelength for P, but 550 nm and 630 nm for N treatment at Z12 growth stages. At Z20 growth stages, there were 420, 500, 680, 750 nm wavelength for P and also 450, 710 nm for N treatment. Likewise, there was no wavelength for P, but 530 nm, 610 nm and 710 nm for N treatment at Z30 growth stages. At Z40 growth stages, there were 450 nm wavelength for P and also 450, 530, 560 and 710 nm for N treatment. There was no shift of wavelength for P, but 520 nm, 570 nm and 710 nm for N

Table 3: Interchange of reflectance at Z12, Z20, Z30, Z40 and Z70 than the control treatment

Growth stage	% reflectance of control than stress	Zero N	Zero P	10% N	10% P
Z12	higher	200−525.5, 626−698.5, 716.5−1100			
	higher	526−625.5, 699−716	200−1100	200−1100	200−1100
Z20	lower	200−452, 709.5−1100	200−1100	200−441, 717−1100	200−427, 505−652, 687.5−742.5
	higher	452.5−709		441−717	427−504, 652.5−687, 743-1100
Z30	lower	200−325, 338−520.5, 608−702	280−322.5, 354−1100		200−458, 511.5−630.5, 698.5−742, 891−1100
	higher	326−337, 521−607, 703−1100	200−279, 323−353	200-1100	458.5−511, 631−698, 743−890.5
Z40	lower	350−450	350−420	350−530, 590−700	350−430
	higher	450−1100	420−1100	700−1100	430−1100
Z70	lower	520−580		520−580	
	higher	350−520, 580−1100	350−1100	350−520, 580−1100	350−1100

Table 4: Shift of percent reflectance in vertical axis of N and P stress plant from the control treatment at different wavelengths (nm) at different growth stages

Growth stage	Phosphorus	Nitrogen
	Wavelength (nm)	
Z12	-	550, 630
Z20	420, 500, 680, 750	450, 710
Z30	-	530, 620, 710
Z40	450	450, 530, 560, 710
Z70	-	520, 570, 710

treatment at Z70 growth stages.

The shifting of the wavelength among the different plant growth stages might be due to the changes in the chemical concentrations at the different levels of stresses. Milton et al. (1991) found that there was horizontal shift of red edge position with the growth stage and it was toward longer wavelength. These results are also confirmed by (Ayala-Silva and Beyl, 2005; Fridgen and Varco, 2004; Li et al., 2006; Mutanga and Skidmore, 2007). The shifts in wavelengths that identified the nutrient deficiencies as the plant matures might also be due to the plant structural changes; the availability and mobility of nutrients in older and younger leaves or the different partitioning of nutrients from vegetative to reproductive growths.

3.6. Change of peak of reflection or absorption

There were different peaks or range of wavelength values at the same wave range for the different level of N and P stressed plants (Table 5). That suggested that in a given wave range of spectrum, N and P nutrient stressed plants had different chemical constituents in the leaves which reflected or absorbed the particular wavelength.

Table 5: Shifting of peak or absorption or wave range wavelength (nm) at different treatment in electromagnetic spectrum on grain filling stage (Z70)

Treatment	Blue	Blue edge	Green	Yellow	Red	Red edge	NIR
Control	439–491	483–495	550, 554	587	674	680–740	760–926
No N	438		551, 556		677		769–778
No P	465		552		675		770, 776
10% N	439–479		554		673		760–896
10% P	439–508		551		676		768–926

4. Conclusion

The reflectance signature changed from UV to NIR and with growth stages from 2 leaf to grain filling stages for plant N and P stresses. The percent reflectance of stressed P and N plants was interchanged at a particular wavelength. These wavelength ranges could be useful to establish the P and N indices. For future research, it could be possible to find out more robust separable wavelengths when N and P were combined and the interaction effects of these two nutrients.

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