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Hyperspectral Imaging Assisted Evaluation of Diverse Crop Residue and Nitrogen Management Practices in Wheat Crop

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Abstract

The study reports our evaluation of high resolution, hyperspectral leaf reflectance and pigment measurement as a potential tool to aid in identifying and delineating the effect of crop residue management and macronutrients on chlorophyll content and crop health of wheat crop (*T. aestivum*). The split-plot design was employed for the experiment with rice straw management practice as the main plots, while 4 sub treatments include an increase in N % from 23 % to 50 % compared to the control. Hyperspectral reflectance data (350-1000 nm) at 5 nm resolution were collected after 2nd irrigation and N % dose application at about 85 days of crop maturity using a SPECIM camera under natural light conditions from ~1200-1500 hrs. The reflectance was measured at ~60 cm from the plant tip and the variance and multivariate mean separation among the various treatments. There was a significant increase (~1.5 fold) in reflectance for the T₄ treatment compared to the control (T₁), and a corresponding increase in chlorophyll content was observed with the T₄ treatment compared to the control. The increase in chlorophyll was also correlated with the content of mineral N soil (mg/kg). With the addition of additional N % along with residue-managed plots, there is a linear increase in chlorophyll content, which is also compared with SPAD and green seeker (NDVI) data taken simultaneously at the time of HSI imaging. This is the first observation where the HSI technique is successfully employed to study the impact of crop residue management on crop health.

Keywords: Crop Residue management; Hyperspectral Imaging; Canopy Reflectance; Wheat; Chlorophyll

Introduction

Wheat is grown in 13 per cent of the cropped area of India. Next to rice, wheat is an essential grain of India and is the staple food of millions of Indians, particularly in the northern and northwestern parts of the country. India is the second largest wheat producer after China, accounting for 14 % of the world's total wheat production (FAOSTAT, 2020). The Rice Wheat Cropping System (RWCS) is India's most widely adopted and practised on 11 million hectares. This system is prevalent in Indo Gangetic Plains (IGP) and is predominant in Uttar Pradesh, Punjab, Haryana, Bihar, West Bengal, Madhya Pradesh provinces etc. In India, wheat occupies an area of about 29.3 mha, with a total production of 103.6 mt and productivity of 3,533 kg ha⁻¹ (FAOSTAT, 2021). RWCS plays a crucial role in food security and, on the contrary, results in several leading problems, including the exhausting nutrient pool in soil, deteriorating soil health, groundwater depletion, escalating production cost, and lower availability of labour. Extensive crop residue burning causes environmental pollution, enhanced greenhouse gas emissions, climatic vulnerabilities, and herbicide resistance in weed species. These are few major threats to its sustainability (Dhanda et al., 2022).

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Rice and wheat are the extensive cereal crops that lead to a heavy depletion of soil nutrients. As mentioned, the problem is further intensified when farmers burn the rice residue left in their fields after mechanized harvesting. The leftover rice residue in the fields interferes with tillage and sowing operations of the successive wheat crop; therefore, farmers usually prefer to burn rice residue. About 2 M farmers in the northwest and some parts of eastern India burn an estimated 23 mt of rice residue yearly (NAAS, 2017). Thus, the residues from the RWCS, especially the rice straw, are challenging to manage timely and cost-effectively.

Various sustainable intensification technologies have been developed to circumvent these challenges. In contrast, *in-situ* incorporation, the mulching and removal of residue with minimum or no tillage are the foremost crop residue management options that reduce irrigation and labour requirements, tillage intensity, and straw burning. Sidhu et al., (2015) stated in the latest developments that the Happy Seeder machine could simultaneously plant wheat crops and mulch rice straw on the field's surface rather than burning and incorporating.

Liability is less in paddy residue mulch regarding nitrogen immobilization and straw mulch, conserving soil and water and suppressing weeds. Hobbs and Gupta (2003) found that zero tillage (ZT) and reduced tillage for the crop of wheat have been progressively followed by the farmers in IGPs in northwestern India, which leads to substantial price savings by minimized use of labour and fuel and early planting heading towards possible benefits in yield, mainly where rice crop harvested late. Traditional methods of evaluating the impact of crop residue management and wheat Nitrogen (N) uptake require the destruction of the plant for chemical and biological analysis. However, these methods are more accurate but invasive, destructive, slow and expensive. Therefore, the testing paradigm has shifted towards hyperspectral imaging (HSI), which is faster, non-invasive, non-destructive, and real-time crop monitoring.

N-partitioning in wheat was affected among pre- and post-anthesis periods due to the difference in N- uptake (Bogard et al., 2010). Meanwhile, grain N content was subjected to two sources mainly. First, during the pre-flowering stage, the N is stored in vegetative organs; second, after flowering, N is absorbed from the soil. The Grain N is remobilized from the soil through roots and senescing canopy tissues (Foulkes et al., 2009). The crop residue management practices affect the plants' ability to regulate the N uptake during growth. Moreover, N uptake efficiency also depends upon the root activity of the plant where the crop residue has direct contact (Hawkesford, 2014). It has been widely reported that for the N status in wheat plants, the canopy (leaf) reflectance is a good indicator because it is also related to chlorophyll (*Chl*) content such as *Chl a* and *b* (Wang Q et al., 2004; Reyniers and Vrindts, 2006; Schlemmer et al., 2013).

Therefore, studying the canopy leaf reflectance of wheat as a real-time indicator of N uptake during the growth period and simultaneously predicts the field information for agricultural production and informs the effect of crop residue management for good-quality wheat (Feng et al., 2008; Saberioon et al., 2014). The canopy leaf reflectance can be non-invasively quantified using the hyperspectral imaging technique. The spectral information obtained from leaf reflectance provides a measure of *Chl* content, which is correlated to the N-uptake efficiency of the plant.

Additionally, the HSI reflects N's spatial and temporal variation during the growing season (Viña et al., 2011; Moharana and Dutta, 2016; Raya-Sereno et al., 2022; Ma et al., 2022). The early diagnosis of N stress could be monitored, allowing us to take remedial measures to manage it. Moreover, using the quantitative spectral information of leaf reflectance through HSI, the crop residue management practices could be evaluated that further affects the grain quality of the wheat crop.

Spectral data obtained from hyperspectral imaging instruments and N content in wheat plants have a non-linear relationship (Campus-Valls et al., 2018). The objectives of the current study were (1) to investigate the wheat N uptake using measurement of leaf reflectance using hyperspectral imaging technique and estimate hyperspectral vegetative Indices (VIs), which were inferred by hyperspectral inversion, (2) to investigate the effect of different crop residue management practices on leaf reflectance and (3) to correlate the leaf reflectance as an indicator of change in chlorophyll content verify with SPAD/Green seeker data and N uptake estimated using chemical analysis method and lastly to explore the major challenges for the use of HSI to evaluate the use of crop residue management practices and how these were related to the N uptake and development of the wheat crop.

Materials and Methods

Plant materials and experimental design

The ongoing experiment (initiated in 2021) was selected for the proposed study on rice–wheat cropping system (RWCS) at the Research Farm, Department of Soil Science, Punjab Agricultural University (PAU), Ludhiana, Punjab. A similar experiment was planned earlier to investigate the long-term impact of different straw management practices on carbon fractions under rice–wheat cropping system (Gupta et al., 2022). In the current study, the impact of the nutrient and the straw management practices was correlated using an advanced hyperspectral imaging technique. The experimental site exists in the central plains of the northwestern state of India, Punjab, with the geographic coordinates of 30° 89 N latitude and 75° 79 E longitude. The region has a sub-tropical, semi-arid condition (with cold winters and hot summers).

Wheat variety PBW 725 was sown in the field (individual plot size of 7.0 X 6.5 = 45.5 m²) during the first week of November 2021. The fertilizer application doses (basal dose of P and K as single super phosphate (16 % P₂O₅) = 26.2 kg P ha⁻¹ and K as a muriate of potash (60 % K₂O) = 25 kg K ha⁻¹) were applied to all treatments. The irrigation schedule for the entire crop growth period included application at ~one week before sowing: 100 mm, and four irrigations of 75 mm each at critical growth stages of wheat crop.

Table 1. Split plot design for experiment for various treatments applied on wheat crop

T ₁ (Conventional Tillage)	T ₂ (Zero Tillage with Happy Seeder)	T ₃ (Conventional tillage + incorporation with mould board plough)	T ₄ (Minimum Tillage with Super Seeder)
N ₁	N ₄	N ₁	N ₂
N ₂	N ₁	N ₄	N ₁
N ₃	N ₂	N ₃	N ₃
N ₄	N ₃	N ₂	N ₄

The experiment included four treatments of crop residue management practices laid out in a split-plot experimental design, as shown in Table 1. Different tillage practices are employed to manage crop residue. The treatment T₁ includes the wheat sowing following conventional tillage (CT) after removing the rice straw. The treatment T₂ includes wheat sowing following the zero tillage (ZT) practice using a happy seeder by retaining rice straw. The treatment T₃ has wheat sowing, where the rice straw was incorporated into the soil using a reversible mould board plough following the conventional tillage practice. Lastly, the treatment, T₄, follows wheat sowing with minimum tillage practice with a super seeder while incorporating the rice straw into the soil. Four sub-treatments were given to crop residue managed plots where timing and concentration of N application to wheat crop was managed. The first sub-treatment (N₁): no-N control- no fertilizer N application, N₂: Recommended practice: 23 % N through DAP at sowing + 38.5 % N as urea at 1st irrigation + 38.5 % N at 2nd irrigation. third sub-treatment (N₃): 50 % N at the time of sowing (broadcast 27 % N through urea before sowing + drill 23 % N through DAP at sowing) + 50 % N through urea at 1st irrigation. Fourth sub-treatment N₄: 50 % N through nitrophosphate (24:24:0) at sowing through drilling + 50 % N as urea at 1st irrigation.

Hyperspectral Imaging Process

Hyperspectral imaging and analysis were utilized as a high-quality phenotyping tool for estimating the N status in wheat plants during the growing season based on spectral information (Figure 1). In this experiment, a portable HSI system was used to collect the leaf canopy reflectance of wheat crops directly in the field. The HSI system consists of a portable hyperspectral imager, a portable stand, a standard white plate for reference spectra and the sun as a light source (Fig. 1a-b). The portable VIS-NIR hyperspectral imager SPECIM (SPECIM, Spectral Imaging Ltd. Finland) was used to acquire the spectral images. It covers the spectral range from 375 to 1000 nm at 5 nm increments for 128 bands, with an image resolution of 690 × 520 pixels. The experiment was performed in natural sunlight from ~1200-1500 hrs.

The hyperspectral data were calibrated and analyzed using the SPECIM Studio IQ software (SPECIM Ltd, Finland). The regions of interest (ROI) were manually selected based on each plot position, and the spectral wavelength data were collected. Hyperspectral imagery was collected after 85 days of sowing (DAS). All the fertilizer treatments were employed and two irrigations, at 25 DAS and 45 DAS were completed. The crop was in the pre-anthesis phase. The data was taken when the natural light was fully available. The background of reflectance data has been corrected from the standard,

while Teflon plate spectral data was collected during every measurement, as shown in the real-time image in Figure 1 b. The spectral information obtained from leaf reflectance using HSI provides a quantitative measure of chlorophyll (*Chl*) content. The spectral information was correlated to the N-uptake efficiency of the plant. In addition, the HSI reflects N's spatial and temporal variation during the growing season. Various spectral indices have been reported to estimate leaf nitrogen content in plant leaves. The three spectral indices were selected having precise physical meaning and high degrees of recognition for comparative analysis and estimation of *Chl* content in this report (Zhu et al., 2008; Hansen and Schjoerring, 2003; Hassan et al., 2019).

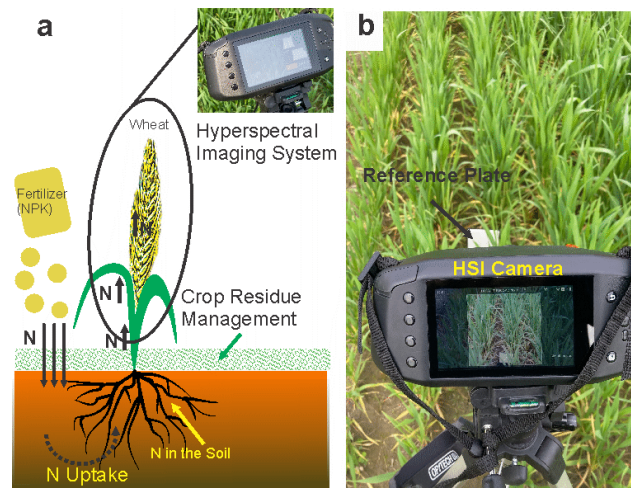


Fig 1. Schematic of hyperspectral imaging systems for evaluating N and crop residue effect from canopy leaf reflectance. Hyperspectral remote sensing can capture information reflecting nitrogen (N) status in wheat plants in real-time and non-destructively.

Table 2. Selected vegetation indices (VIs) that have been applied to wheat under field conditions

Number of Bands	Vegetative Indices (VI)	Formulation	Reference
Two	Normalized Difference Vegetation Index (NDVI)	$R_{790} - R_{660} / R_{790} + R_{660}$	Hansen and Schjoerring (2003) Hassan et al. (2019)
Two	RVI - 870/660 Ratio Vegetation Index	R_{870}/R_{660}	Zhu et al. (2008)
Two	RVI - 810/660 Ratio Vegetation Index	R_{810}/R_{660}	Zhu et al. (2008)

Periodic mineral N (NO_3 and NH_4) in soil and plant N uptake

Soil samples were collected (0-15.0, 15-30 and 30-45 cm depths) at sowing, 25-30, 50-55 days after sowing and at maturity for determining NO_3 -N and NH_4 -N. 10 g portion of fresh soil samples was extracted with 100 ml of 2 M-KCl solution after shaking for one hour. Then, the suspension was filtered, and filtrated aliquot was investigated for NO_3 -N and NH_4 -N by steam distillation using Devarda's alloy and MgO, respectively.

Simultaneously, wheat plant samples were collected to determine dry biomass accumulation and plant N uptake at the crown root initiation stage (CRI), maximum tillering stage (MTS), flowering and maturity stage. The data for mineral N from soil and N uptake was discussed in correlation to the increase in canopy leaf reflectance as an indicator for an increase in *Chl a* and *b* content.

Chlorophyll content measurement with SPAD meter

To determine the crops' chlorophyll content, Soil Plant Analysis Development (SPAD) chlorophyll meter readings were recorded using a Minolta SPAD-502 chlorophyll meter. Using the SPAD meter, the uppermost fully expanded leaf of 10 randomly selected plants was measured for chlorophyll content. The data was recorded from the dry surface of the insect-pest-free leaves.

Green seeker optical sensor

The Green Seeker TM handheld optical sensor unit Model 505 was used to measure the Normalized difference vegetative index (NDVI) from the crop canopy. The NDVI readings taken by Green Seeker and the following equation calculated the NDVI values

$$NDVI = (NIR_{ref} - RED_{ref}) / (NIR_{ref} + RED_{ref}) \dots\dots [1]$$

NIR ref and Red ref correspond to reflectance in the near-infrared and red bands. The eventual grain yield can be predicted mid-season using the NDVI to measure total biomass and leaf greenness.

Results and Discussion

Hyperspectral imaging (HSI) of wheat canopy leaf

HSI-captured data corresponds to three-dimensional (3D) hypercube consisting of two spatial dimensions (X and Y axis) and one spectral (wavelength (λ)) dimension (Gowen et al., 2007). It captures multiple images at different wavelengths ranging from 400 – 1000 nm for the same spatial area. HSI provides a large amount of data that helps analyze the target's inherent properties, including micronutrient uptake by plants from soil. When crops/plants are exposed to natural light (Spectrum), they either reflect, scatter or absorb in a unique pattern at respective wavelengths due to their chemical composition and inherent physical structure. This pattern was referred to as a spectral signature or spectrum. We have captured the spectral signatures of wheat crop sown in Nov, 2021 using a handheld SPECIM hyperspectral imaging system after 85 days of sowing over the four main treatments of crop residue management (T_1 , T_2 , T_3 and T_4) with four sub-treatments for each main treatments (N_1 , N_2 , N_3 and N_4). In four main sub-treatments, the % N employed to the crop was varied from No nitrogen, 38.5 % N (RDF) to 50 % N (N_3 and N_4).

The crop canopy has strong absorption and reflection characteristics in the visible and near-infrared bands, which are related to the physiological and biochemical components of the crop (Strachan et al., 2002). Several recent reports illustrated that the canopy spectral reflectance vegetative Indices (VIs) in wheat plants was an indicator of N (Hansen and Schjoerring 2003, Zhu et al., 2008; Liang et al., 2018; Hassan et al., 2019). When natural light hits a wheat plant, most of the irradiance was consumed by water transpiration, and a small portion was used for CO_2 assimilation. Partially, the canopy absorbs the light energy and reflects a part to the space. The chlorophyll content influences the reflected light in the visible spectral region in the wheat canopy, which is related to the concentration of leaf N (Thomas and Gausman, 1977; Wessman, 1990). Chlorophyll strongly absorbs in the spectral range from 450 – 670 nm and reflects strongly in the green light, correspondingly having reflectance in the infrared red (0.7- 1.3 μ m) region. There was an increase in normalized leaf canopy reflectance (700 nm- 900 nm) by 1.5 fold within N sub-treatments ($N_4 > N_3 > N_2$) to the wheat crop as compared to the control (N_1) (Fig 2 a-d). The reflectance varies from 0.5 to 0.85. Figure 2 (a-c) illustrates that, with the increase of nitrogen content in a leaf canopy, the leaf canopy spectral reflectance increased significantly in the near-infrared region (>700 nm) and decreased in the visible region (400 – 700 nm) as compared to the control where no nitrogen fertilization was applied (T_1). The phenomenon is consistent with our leaf canopy reflectance findings and agrees with the recent reports (Liang et al., 2018). When fertility or the applied nitrogen content is sufficient, crops are usually denser and have higher photosynthetic activity, and *vice versa*, intensely absorb more in the visible region and have higher reflectance in the near-infrared region. Additionally, at the same growth stage, the leaf nitrogen content of wheat increased with the increment in nitrogen application rate, but the increase became steady eventually at the higher nitrogen application rate, for instance, 150 kg/ha.

The Vegetative Indice(s) (VI), which are derived from wheat canopy hyperspectral reflectance, are used to describe vegetation characteristics that depend on the environment. The list of indices in Table 2 summarizes 3 VIs that partially provide information on the N status of the entire wheat plant under field conditions (Hansen and Schjoerring, 2003; Hassan et al., 2019, Zhu et al., 2008). VIs for predicting *Chl* contents are usually based on (i) reflectance values far from the pigment absorption maxima and (ii) the selection of wavelengths close to the absorption bands. Another exciting region of the spectral area is the region between the strong red light absorption by *Chl* (680 nm) and the highly reflective near-infrared wavelengths (780 nm), a region of the spectrum known as the "red edge;" such as RVI 810/680 or RVI 870/680 (Zhu et al., 2008).

Two-band VIs were primarily used for N estimation (Table 2), namely NDVI (Normalized differentiated vegetative index). VIs are calculated from canopy reflectance values extracted from the HSI spectra for specific visible (660 nm) and near-infrared wavelengths (790 nm) (Frels et al., 2018). NDVI allows us to estimate the changes in canopy *Chl* content and thus indicates the N status of wheat plants (Gutierrez et al., 2004). The NDVI $(\lambda_{790} - \lambda_{660})/(\lambda_{790} + \lambda_{660})$ was estimated from the canopy leaf reflectance data shown in Fig 2. (a-d). There is a non-linear increase in the NDVI with an application of N from N_1 to N_4 and crop residue management practices (Fig 3c). In all the treatments, the NDVI values between 0.8- 0.9 indicate the healthiness of wheat plants in comparison with N_1 (T_1) control. A significant difference was observed for crop residue

management practices compared to the control plots. With the increase in N %, the NDVI values show an increase in the case of T₃ and T₄ treatments. The most promising results were obtained with conventional tillage besides straw incorporation with mould board plough (T₃) employing 50 % N in 24:24 ratio at the time of sowing and 50 % N at 1st Irrigation (Liang et al., 2018).

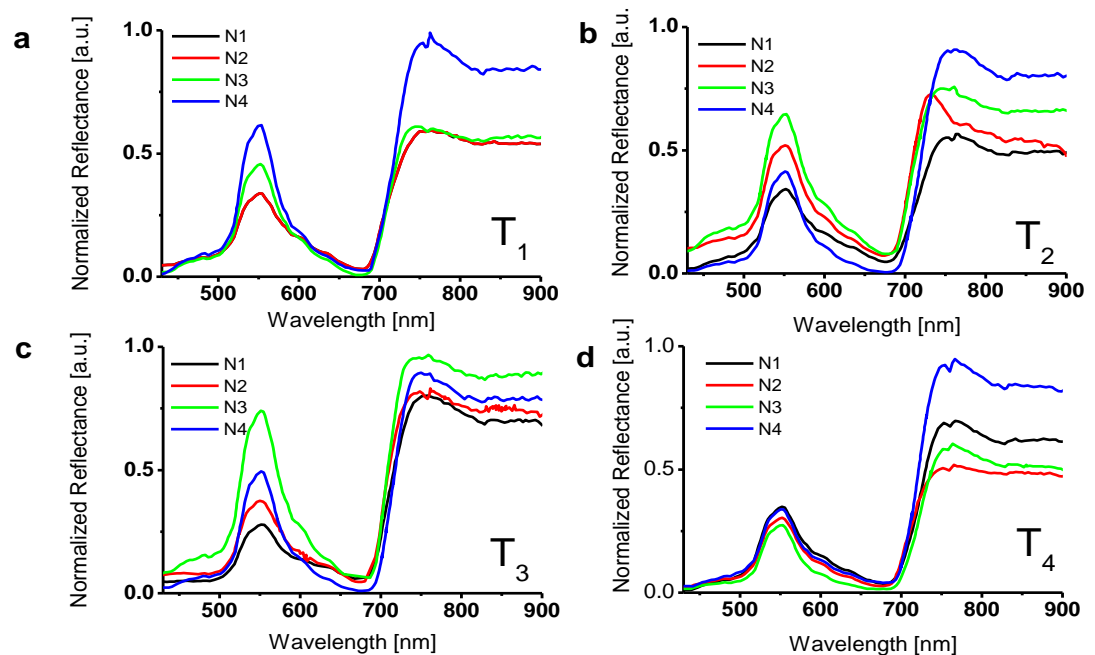


Fig 2. The normalized canopy leaf reflectance of wheat crop averaged over ROI (1 x 1 m²) (a) T₁, Conventional Tillage N₁ with N-Control and No Fertilizer application, N₂, Recommended practice, N₃ 23 % N+ 38.5% N+ 38.5% N, N₃, 50% N (23 % before sowing + 27% at sowing) + 50 % N (at 1st Irrigation) and N₄, 50 % N (24:24) + 50 % (at 1st Irrigation) (b) T₂, Zero tillage + sowing with Happy seeder by retaining rice straw, (c) T₃ Conventional Tillage + Rice straw incorporation with MB Plough S, (d) T₄, Minimum tillage wheat sown with super seeder.

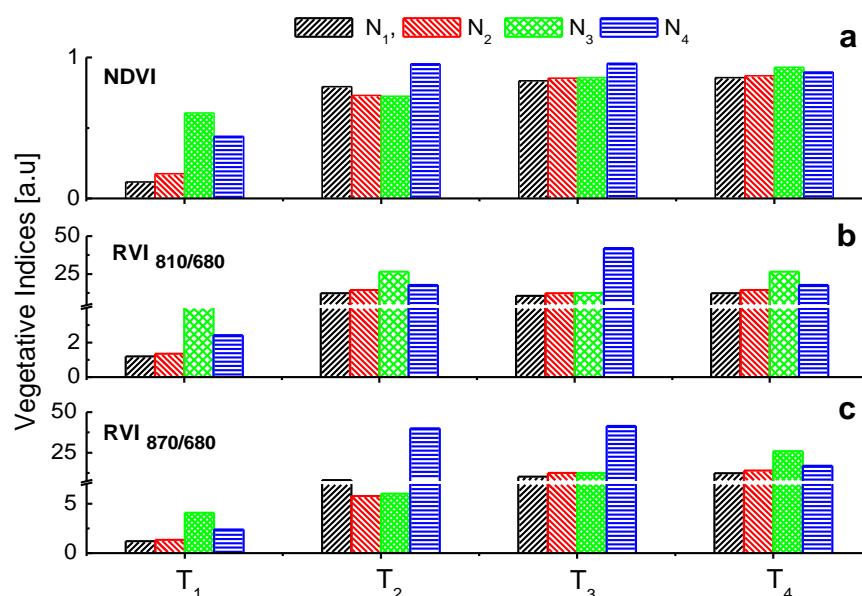


Fig 3. Variation in Vegetative Indices (VIs) amongst different crop management practices (T₁ (Control), T₂, T₃ and T₄) and N application (s) (N₁, N₂, N₃ & N₄) to wheat crop as an indicator of Chl content from canopy leaf reflectance. (a) Normalized Vegetative Index (NDVI) (b) RVI-810/660 (Ratio Vegetation Index) (c) RVI -870/660.

Zhu et al., (2008) identified typical spectral bands and VIs to characterize the N status of wheat leaves and analyze the quantitative relationship between leaf N status and canopy reflectance. The ratio VIs (RVIs) (R870, R660) and RVIs (R810, R660) showed the highest correlation with leaf N status compared to other specific RVIs (Zhu et al., 2008; Liang et al., 2018). Therefore, the RVIs 810/660 nm and 870/660 nm were determined, as shown in Fig 3 b – c. The RVIs for T₃ treatment (conventional tillage and incorporation with MB plough) were several-fold higher than T₁ crop residue

management practices. Higher values of RVIs illustrate higher *Chl* contents in leaves, correspondingly higher N uptake (Zhu et al., 2008).

Mineral N (NO_3 and NH_4) in soil and plant N uptake

To confirm the predictions of indications from spectral data, the estimation of periodic mineral N (NO_3 and NH_4) was carried out under lab conditions. In all four crop residue management practices and N applications, there is 1.3 fold increase in mineral N compared to the control. This indicates the retention of N in soil with straw management practices. The top soil layer (0–15 cm), where rice residue was incorporated using an MB plough three weeks prior to wheat sowing (T_3), recorded maximum soil nitrate-N content of 25.4 mg/kg after 25 days after sowing (DAS). Also, the T_3 treatment at 45 DAS level exhibited maximum NO_3 -N content (20.1 mg/kg) at a further increased soil depth of 15–30, 30–45 and 45–60 cm, respectively (data not included). However, retaining rice residue with happy seeder (T_2) might have improved the activity of nitrifying bacteria, promoting the nitrification process and thus rapidly converting NH_4^+ into NO_3^- thereby contributing to the highest soil nitrate-N content (20.5 mg/kg. Chen et al., (2017) reported that plough tillage with straw incorporation management significantly increased the soil NH_4^+ -N and NO_3^- -N contents in the 0–30 cm layers, which were also revealed in the current study for the NO_3^- -N content. Moreover, conventional tillage (CT) practices (T_1) had a profound effect in exhibiting maximum nitrate-N concentration at various soil depths (15–30, 30–45 and 45–60 cm) at 25 DAS. CT was shown to break the plough pan layers, reduce the penetration resistance and improve rainfall interception and soil nutrient cycling (Essel et al., 2019), thus increasing soil NO_3 -N content in the 20–50 cm layers.

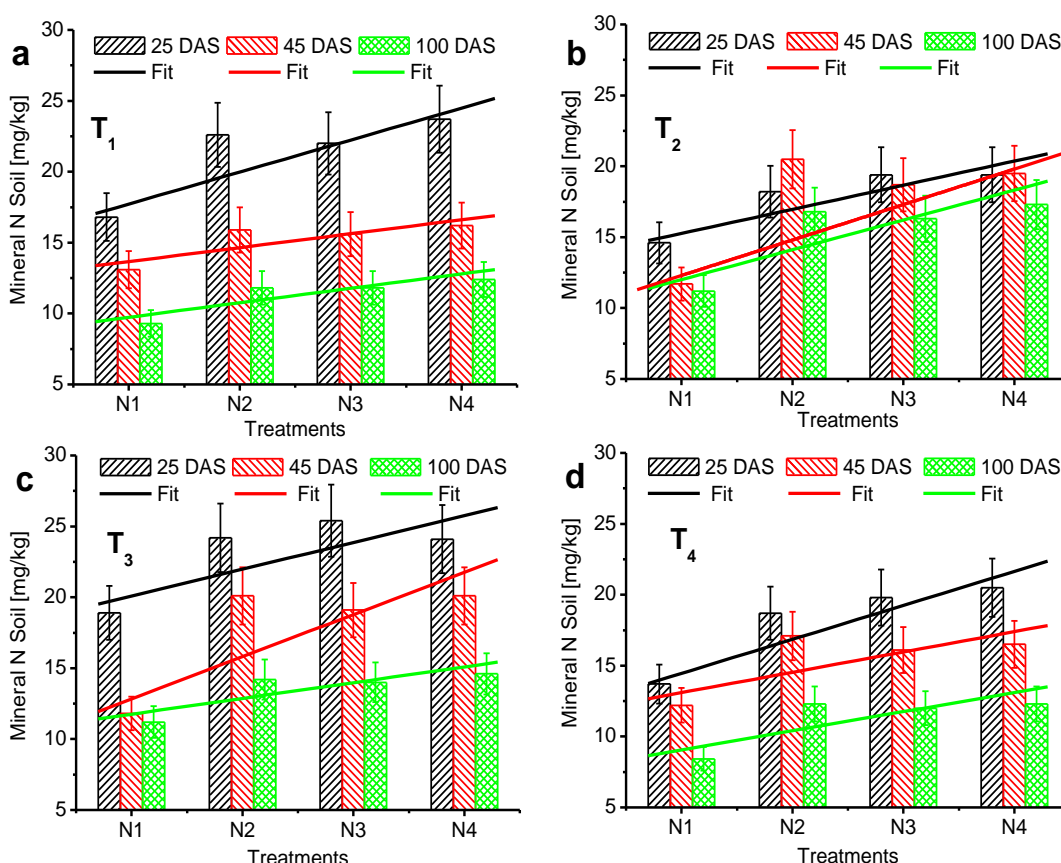


Fig 4. Mineral nitrogen (N) soil (mg/kg) 25, 45 and 100 days after sowing (DAS) for different crop residue management practices with four different N sub-treatments in wheat crop. (a) T_1 (Control) (b) T_2 (c) T_3 and (d) T_4 . Black, red and green lines indicate the linear fit for individual mineral N Soil (mg/kg) at 25, 45 and 100 DAS, respectively. For all four crop residue management practices, the T_3 treatment retains higher mineral N soil (mg/kg).

Arora et al., (2010) reported that surface retention of residue and zero tillage potentially persuade major transformations in the dynamics of N and management of N compared to tillage and straw removal. While zero tillage may diminish the mineralization of N by reducing the breakdown of soil organic matter, within 3–4 years of its adoption, the crop straw can affect the dynamics of N from volatilization and immobilization (Singh et al., 2005). Though, with surface retention of residue (Xu et al., 2010), nitrogen broadcasting onto the residue of the crop can be a wasteful application method because of the immobilization in connection with microbial breakdown of rice straws and

because of higher ammonia volatilization, than when, applied to the bare soil (Patra et al., 2004). Apply only a meagre nitrogen amount with seed and postpone most top dressing N until after the hefty decomposition of crop residue has occurred; then, irrigation application is one potential solution to such problems.

Figure 5 represents the percent N uptake by the wheat crop as a function of different crop management practices (T_1 to T_4) and incremental % N application (N_1 to N_4). With the increase in N application besides residue management, there was a linear increase in % N uptake by the plants. T_3 treatment exhibits the maximum % N uptake after 25 DAS (~ 8%). As discussed earlier, the rice residue incorporated using an MB plough three weeks before the wheat sowing (T_3) shows maximum N retention at 25 DAS; thus, % N uptake by the wheat crop was also higher or linearly correlated. Percent N uptake reduced at 45 and 100 DAS, although it shows a linear increase with increment in N applications. The variation for % N uptake amongst different crop management practices shows no significant difference.

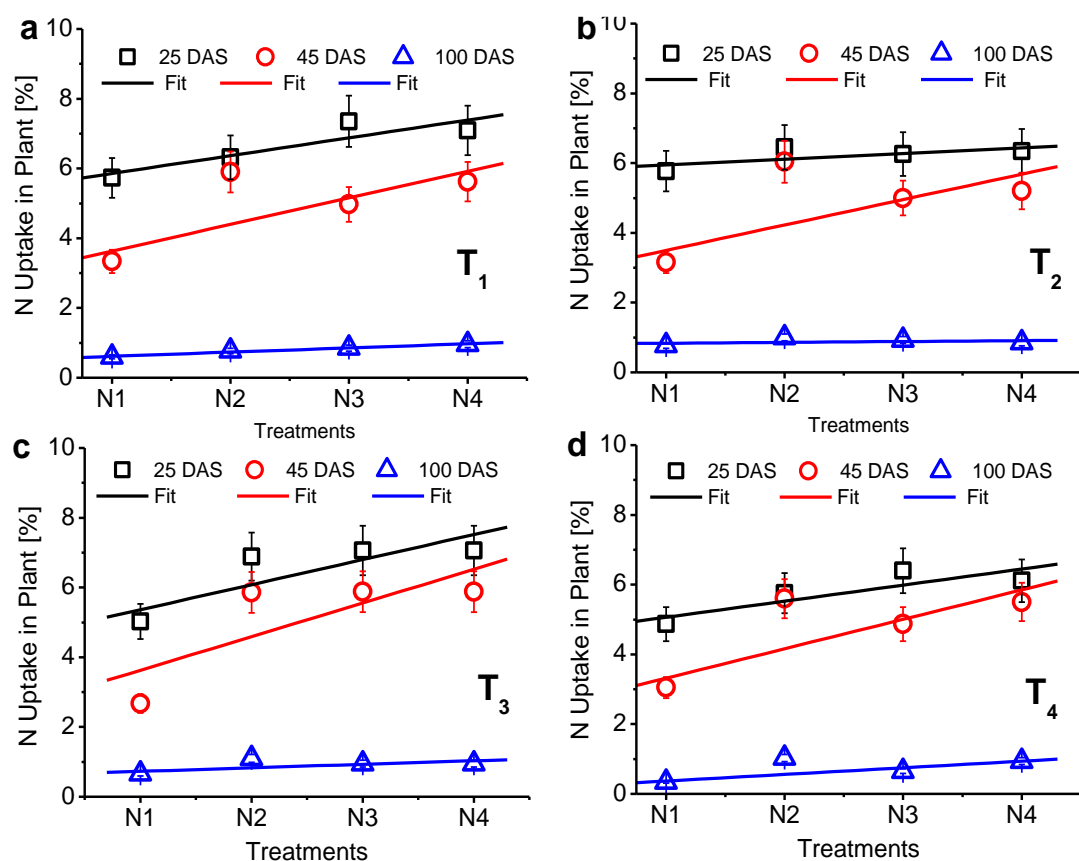


Fig 5. Percent N uptake by wheat plants as a function of N application (N_1 to N_4) and crop management practice (T_1 to T_4). Black, red and blue lines represent the linear fit for all four treatments.

Chlorophyll content measurement with SPAD meter

The relationships between leaf (*Chl*) and SPAD values were non-linear for all the treatments (Fig. 6). The slope of the relationship between (*Chl*) and SPAD increased initially with increasing SPAD, and saturated with N applications on wheat crop. Higher SPAD values indicate a higher N uptake. The data fitted well to the 2nd-order polynomial function with two slopes, and r^2 values were ~0.9 for all the treatments. SPAD values varied from 42- 45 for all the N sub-treatments (N_2 , N_3 , N_4) and were 1.2 times higher than the control N_1 in all the crop residue management practices (T_2 , T_3 , T_4). All the treatments in this study had non-linear SPAD–(*Chl*) relationships with the increasing slope with increasing SPAD values (Fig. 6 a-d). Most studies that quantify the relationship between (*Chl*) and SPAD values employ linear regression (Xu et al. 2000; Yamamoto et al. 2002; Esposti et al. 2003; Kapotis et al. 2003; Murillo-Amador et al. 2004; Wang et al., 2004). Linear regressions between (*Chl*) and SPAD determined for the present data sets resulted in lower r^2 values (*data not shown*) and a systematic pattern of the residuals, with under predictions of (*Chl*) in the low and high SPAD ranges. The effect of non-uniformly distributed chlorophyll amongst all the treatments is likely to be more important in explaining the nonlinearity in the empirical relationships since the scattering effect was predicted to be comparatively weak.

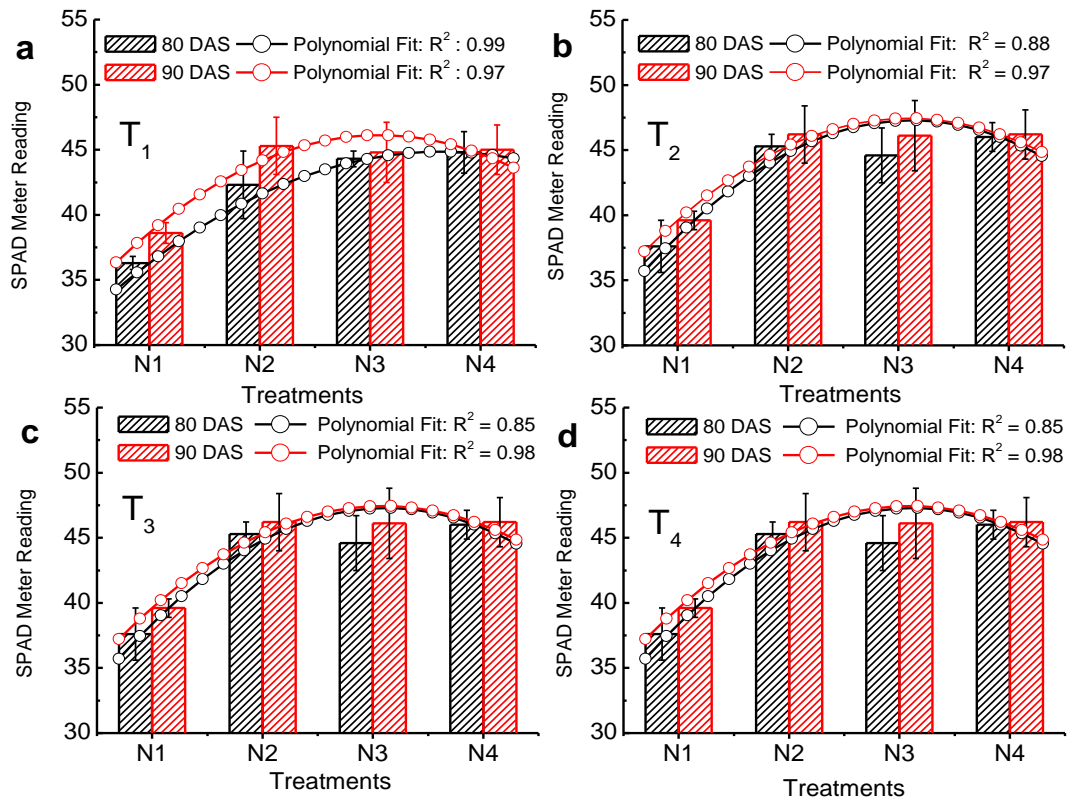


Fig 6. SPAD meter estimating the chlorophyll content for the wheat crop after 80 and 90 DAS. Red and black circles with lines represent the second-order polynomial fit with r^2 values 0.85 to 0.98. Non-linear increase in the SPAD values with an increase in N application.

Green seeker for real-time NDVI measurement

Fig. 7 represents NDVI estimated in real-time using a Green seeker optical sensor. Treatments recorded significantly higher green seeker values and correspondingly higher N uptake for the entire N schedule than control. In all the crop residue management practices (T₂, T₃ and T₄), the NDVI index (0.7- 0.8) remains higher than the control (T₁) (0.5).

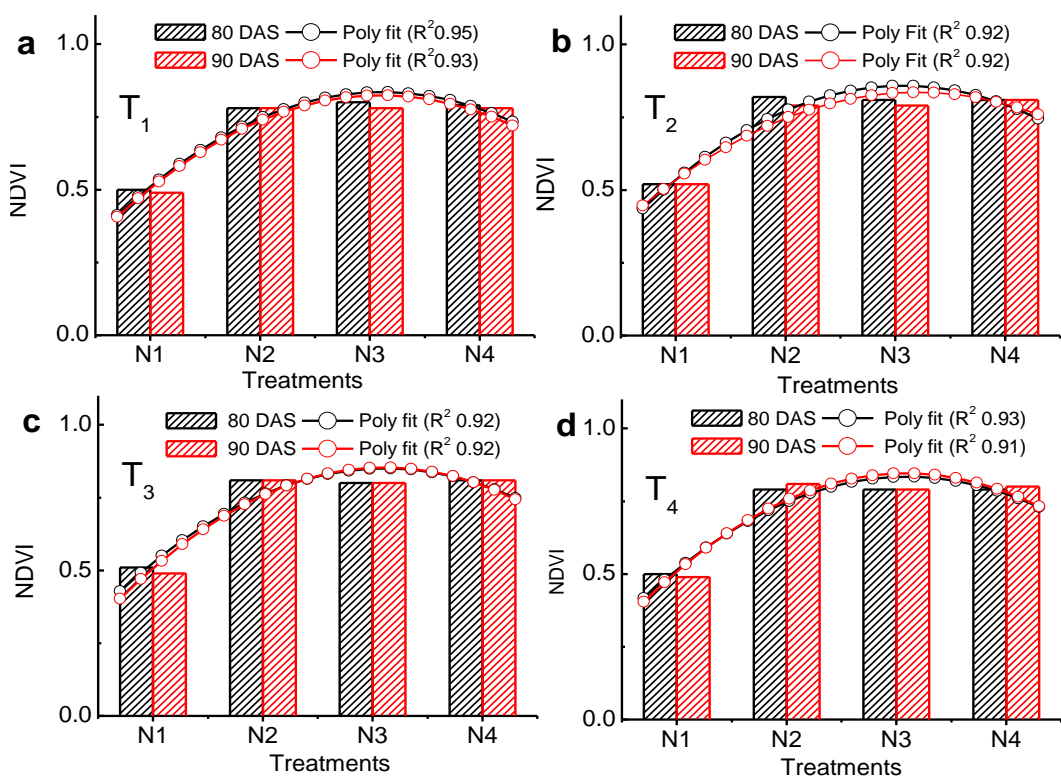


Fig 7. NDVI estimation of wheat canopy leaf reflectance using Green Seeker Optical Sensor. There was a non-linear increment in the NDVI values obtained using the green seeker sensor. Black and red lines indicate the second order polynomial fit having r^2 values higher than 0.93.

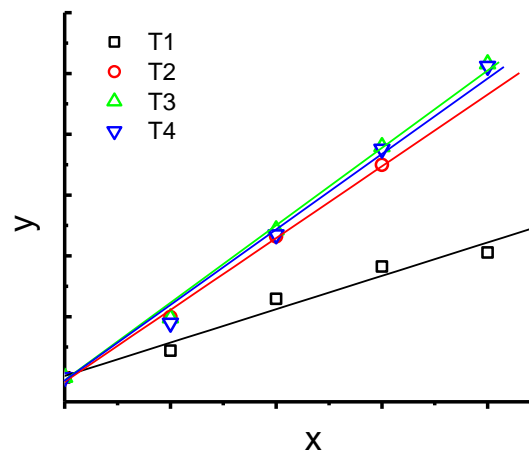


Fig 8. Correlation plot between NDVI values obtained from the analysis of hyperspectral imaging spectra and real-time NDVI estimation using Green seeker optical sensor after 80 DAS.

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}} \dots\dots\dots (2)$$

Green seeker values indicate that the % N uptake amongst all the treatments remains higher than the control rather than having no signs of N addition. The NDVI values estimated from hyperspectral imaging have a linear relationship with NDVI estimated using Green seeker. The correlation plot indicates a strong relationship between the NDVI estimated from HSI technique and real-time NDVI measured using Green Seeker. Figure 8 illustrates a strong correlation NDVI at 80 DAS and simultaneously measuring HSI in the same week. The Pearson correlation coefficient (r) estimated by the equation (2) where x and y are variables indicating experimental and SPAD NDVI values, respectively. The r value for T_1 , T_2 , T_3 and T_4 treatments, 0.7576, 0.9069, 0.9469 and 0.9456, respectively, indicate strong positive correlation, which means that high X variable (NDVI_HSI Camera) scores go with high Y variable (NDVI_SPAD) scores (and *vice versa*). The crop maturity was at the same stage. The non-invasive and no destructive mode of estimation of NDVI using HSI system have the potential to estimate the % N uptake nearly in agreement with wet lab experiments.

Conclusion

An accurate assessment of the in-plant N status of wheat is essential for predicting grain protein content and composition, in addition to ensuring food and nutritional safety. We are beginning a promising path toward using hyperspectral imaging systems to predict the effect of N application and crop management practices in wheat plants. We determine a positive correlation between the real-time data acquired through the HSI system and lab estimation of N uptake and mineral N soil. On the one hand, as a high-resolution imaging spectral collection tool, hyperspectral imaging and sensing technology can potentially complement or even replace a few field measurements for some wheat N-related traits in the growing season. Nevertheless, combining hyperspectral VIs and machine learning algorithms could be a powerful tool for estimating agricultural indices from hyperspectral remote sensing data.

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Abbreviations

HSI – Hyperspectral Imaging, IGP, Indo-Gangetic Planes, VI- Vegetative Index

Author Contributions

The original idea of research was conceived by RKG and MSS. The field experiments and data collection are jointly done by VS and MSS. Data analysis was done by MSS, VS and RKG. The manuscript is written and results were discussed by MSS, VS and RKG.

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Competing interest

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Ethics approval

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