

# Parametric estimation of net photosynthesis in rice from *in-situ* spectral reflectance measurements

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**Net photosynthesis rate ( $P_N$ ) has an important role in controlling the ecosystem primary production and it is important to understand the relationships between  $P_N$  and other physiological and environmental variables to improve and develop models for predicting plant growth and productivity. Here we study utility of ground-based hyperspectral measurements in estimating net photosynthesis during the growth cycle of rice. Ground-based hyperspectral reflectance and micro-meteorological data ( $P_N$ , chlorophyll concentration index (CCI), photosynthetically active radiation (PAR)) were collected from a rice field on three dates in the kharif season at Meerut, Uttar Pradesh, India. Green chlorophyll index ( $CI_g$ ) showed a positive and statistically significant relationship with CCI ( $R^2 = 0.64$ ). Use of single index showed poor fit with  $P_N$ . The model based on a combination of two variables (vegetation index (VI)  $\times$  PAR) however proved better in explaining the variance of  $P_N$ . Among the VI-based combinations comprising three variables which depend on the Monteith light-use efficiency (LUE) concept, models consisting of either  $CI_g$  or CCI as one variable appeared to be the best in predicting  $P_N$  with  $R^2$  of nearly 0.6. The study concludes that a single index is insufficient to account for factors controlling variation in  $P_N$  estimation whereas Monteith logic-based combinations have shown its potential for  $P_N$  estimation by incorporating the chlorophyll based and other vegetation indices as the proxy of LUE and  $fAPAR$  (fraction of absorbed photosynthetically active radiation) respectively.**

**Keywords:** Hyperspectral measurements, LUE model, net photosynthesis, rice, spectral indices.

KNOWLEDGE of carbon uptake via photosynthesis in terrestrial ecosystems has been a subject of increasing interest because of its direct importance to plant growth assessment, national carbon budgeting and mitigating anthropogenic causes of climate change. The rate at which an ecosystem captures and stores chemical energy is called net photosynthesis rate ( $P_N$ ). Understanding the functional relationship of  $P_N$  with other physiological and

environmental variables is crucial to improve and develop new models for crop productivity estimation and ecosystem carbon management. Rice is the most staple food crop in Asia and accounts for 11% of global crop land area<sup>1</sup>. Given the doubling of rice demand and projected water-scarcity effects under future climate on rice production in Asia<sup>2</sup>, research strategy should focus on methods to improve crop models and to better predict crop yields for achieving food security and sustainability.

In the past, several laboratory studies have attempted leaf-level measurements of  $CO_2$  exchange and net photosynthesis as a basis for varietal screening and crop improvement<sup>3</sup>. Remote sensing of leaf to landscape scales of crop physiology now provides an opportunity to integrate with leaf-level measurements and has immense potential for timely assessment of primary productivity and source-sink relationships. Measurements of carbon exchange at plot-landscape level with eddy covariance technique and developing satellite-based spectral models for gross primary productivity (GPP) and net primary productivity (NPP) over India have been demonstrated<sup>4</sup>, here we investigate measuring  $P_N$  at leaf level and use handheld spectral measurements to develop a leaf-level approach.

The fundamental methodology of remote sensing-based GPP estimation depends on the light-use efficiency (LUE) concept proposed by Monteith<sup>5,6</sup> as follows

$$GPP = fAPAR \times PAR \times LUE. \quad (1)$$

The product of the incident photosynthetically active radiation (PAR) and the fraction of absorbed photosynthetically active radiation ( $fAPAR$ ) represents the amount of solar radiation absorbed whereas LUE shows efficiency with which the absorbed PAR is converted into biomass. LUE models based on remote sensing have been extensively used in estimating GPP or NPP at the ecosystem level, but they require accurate measurement of PAR,  $fAPAR$  and LUE factor. To some extent, PAR and  $fAPAR$  could be estimated accurately by modelling and remote sensing; however, the ecosystem-level LUE is difficult to measure and causes substantial errors in GPP estimation.

However, whole radiation absorbed by the canopy is not used for photosynthesis. The spectral reflectance from

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the canopy gives the estimation of the green portion of the plant, which makes the remote sensing data useful for quantification of productivity with the help of vegetation indices (VIs). VIs offer important and convenient measures for the estimation of ecosystem variables (e.g. chlorophyll (Chl) content, leaf area index (LAI) and  $fAPAR$ ). Leaf chlorophyll content has a direct impact on the nitrogen content of plants, which in turn determines the photosynthetic activities and amount of PAR absorbed by photosynthetically active vegetation. Peng *et al.*<sup>7</sup> found a positive correlation between LUE and chlorophyll content, which is mainly attributed to the enhanced electron transport activity caused by leaf pigment system<sup>8,9</sup>. VIs like the photochemical reflectance index (PRI) directly indicate the photosynthesis reactions by using two wavebands: 531 nm, affected by de-epoxidation of violaxanthin to zeaxanthin and 570 nm, which remains unaffected by the de-epoxidation reaction<sup>10</sup>, and as a result it has been successfully employed to detect variations in photosynthetic efficiency at the leaf level<sup>11–13</sup>. Among the other VIs, the normalized difference vegetation index (NDVI) is intensively used, but has limitations like its sensitivity to atmospheric aerosols<sup>14</sup>, whereas enhanced vegetation index (EVI) uses extra blue band to take this limitation into account.

These results provided the basis for estimating production by a combination of such indices and climate variables<sup>15–19</sup>. Based on the work of Inoue *et al.*<sup>20</sup>, a new model incorporating VI was proposed to estimate GPP in wheat using MODIS images and PAR<sup>21</sup>. Besides, both LUE and LAI can be estimated by certain VIs<sup>17,20,22</sup>, the latter have linear and robust relationship with  $fAPAR$ <sup>23,24</sup> and  $P_N$  can be estimated either using single index or by the various combinations of VIs and environmental variables in the following equations:

$$P_N \propto VI, \quad (2)$$

$$P_N \propto VI \times PAR, \quad (3)$$

$$P_N \propto Chl \times PAR, \quad (4)$$

$$P_N \propto Chl \times VI \times PAR, \quad (5)$$

$$P_N \propto VI \times VI \times PAR. \quad (6)$$

This article presents a study using two methods for GPP estimation from the *in-situ* measurements during the growth cycle of rice. The objectives of the study are: (1) to evaluate the relationship between ground-based chlorophyll content and chlorophyll-related VI; (2) to evaluate the four kinds of VIs for net photosynthesis estimation, and (3) to identify the most suitable VI-based combination for GPP estimation following the Monteith logic (GPP as a product of LUE,  $fAPAR$  and PAR). The model

will be useful for the development of new approaches of GPP estimation with remote sensing inputs.

## Material and methods

### Site characteristics

The study was undertaken on a rice (*Oryza sativa* L.) field in the experimental farm of the Sardar Vallabhbhai Patel University of Agriculture & Technology (SVBPUA&T), Meerut (29°05'35.1"N, 77°41'52.2"E), Uttar Pradesh, India. This experimental station lies within the sub-tropical zone with an average rainfall of 810 mm. The rice field comprised agronomic experimentation with three types of nutrient treatment (control, 75% NPK with 10 tonnes/ha FYM and 100% NPK). The Pusa basmati variety of rice was transplanted on 13 July 2010, with a sufficient and regular water supply in 2010.

### Hyperspectral reflectance measurements and spectral vegetation indices

Canopy reflectance spectra were obtained under clear sky conditions near mid-day using a portable spectroradiometer (FieldSpec-FR, ASD) on three days (17 and 29 September and 14 October) in 2010. Spectral range and the field-of-view of the sensor were 350–2500 nm and 25° respectively. Inbuilt software (RS<sup>3</sup>) was used to derive the spectral reflectance as the ratio of reflected radiance to incident radiance estimated by a calibrated white reference (Spectralon, Labsphere). Spectral measurements were taken at nadir-looking position at the height of 1 m over the canopy. Reflectance measurements were taken by averaging 10 scans at optimized integration times. Radiometric data were collected close to solar noon (between 11:00 and 13:00 local time), when changes in solar zenith angle were minimal. Spectral measurements were used to compute four VIs, namely EVI, NDVI, scaled photochemical reflectance index (sPRI) and green chlorophyll index ( $CI_g$ ) as listed in Table 1 (refs 25–28).

### Net photosynthesis and PAR measurements

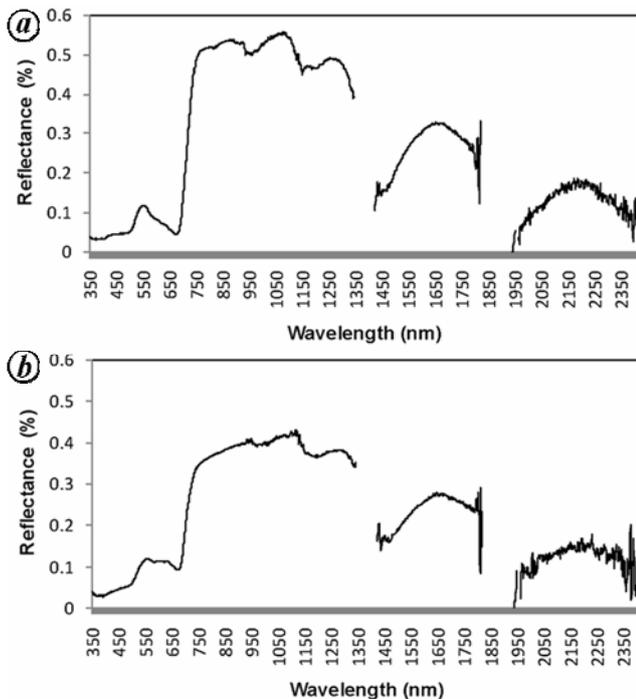
$P_N$  was measured using a CI-340 portable gas analysis system (CID Biosciences, Inc.) with a quantum sensor in the measuring cell to obtain PAR. This instrument also gives simultaneous measurements of leaf and air temperatures. These measurements and reflectance data collection were made during mid-day on each operational day.

### Chlorophyll measurements

A chlorophyll content meter (CCM-200, Opti-Sciences, Inc.) was used to estimate the chlorophyll content in the

**Table 1.** Four different hyperspectral vegetation indices (VIs) used for estimation of net photosynthesis rate ( $P_N$ )

VI	Formula
Enhanced vegetation index (EVI) <sup>25</sup>	$2.5 \times (R_{800} - R_{670}) / (1 + R_{800} + 6 \times R_{670} - 7.5 \times R_{450})$
Normalized difference vegetation index (NDVI) <sup>26</sup>	$R_{800} - R_{680} / R_{800} + R_{680}$
Scaled photochemical reflectance index (sPRI) <sup>10,27</sup>	$((R_{531} - R_{570}) / (R_{531} + R_{570})) + 1) / 2$
Green chlorophyll index ( $CI_g$ ) <sup>28</sup>	$(R_{800} / R_{550}) - 1$

**Figure 1.** Rice canopy reflectance spectra collected around solar noon at (a) anthesis phase and (b) senescence phase.

field from the same leaf used for the measurement of photosynthesis. The CCM-200 uses absorbance to estimate the chlorophyll content in the leaf tissue and calculates a chlorophyll concentration index (CCI) value that is proportional to the amount of chlorophyll in the sample. Two wavelengths (653 and 931 nm) were used for absorbance determination. The first wavelength (653 nm) falls within the chlorophyll absorbance region and the second (931 nm) provides the compensation for leaf structural differences.

## Results and discussion

### Rice canopy hyperspectral signatures

The canopy reflectance of sunlit leaves was always higher than 0.5 in the NIR range at anthesis stage and it was lower than 0.5 in the NIR region in the senescence phase. The absorption peak in the red region was also sharper in the anthesis phase than in the senescence phase (Figure 1).

### General characteristics of photosynthetic parameters

$P_N$  is the accumulated expression of the status of chlorophyll content, environmental stress and incident PAR. In the study, higher magnitude of  $P_N$  at the anthesis stage was the result of higher chlorophyll content and incident PAR. The lower chlorophyll content resulted in lower LUE, which finally caused the reduction in  $P_N$  at the senescence phase (Table 2). The environmental stress was also lesser over leaf at the anthesis stage due to negative temperature difference between leaf and air, which resulted in higher  $P_N$ , whereas higher stress during the senescence phase reduced the  $P_N$ .

### Relationship between CCI and VI

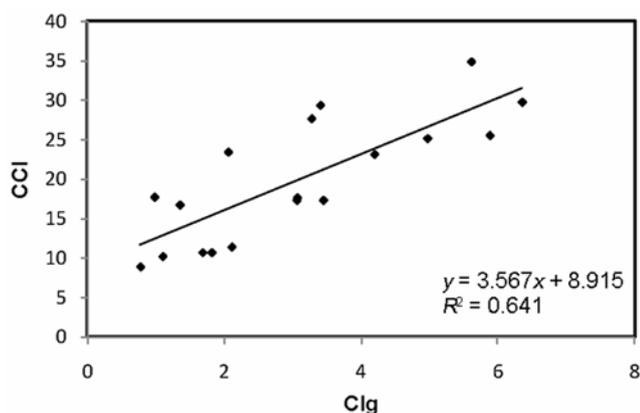
Broadband indices (e.g. NDVI and EVI) have been found sensitive to canopy-scale  $fAPAR$ , but fail to separate contribution from photosynthetic and non-photosynthetic components for accurately estimating the photosynthesis rate. Chlorophyll content is a main factor that influences the amount of PAR absorbed by photosynthetically active vegetation (PAV) and closely related to  $fAPAR_{green}$ . Relationship between chlorophyll content and the pigment-sensitive VIs has therefore assumed significance in predicting magnitude of photosynthesis. We examined this relationship between CCI as a proxy for chlorophyll content and green chlorophyll index ( $CI_g$ ) as the most sensitive chlorophyll-related VI identified for crop canopies<sup>21,29</sup>. Figure 2 illustrates that  $CI_g$  shows a positive and statistically significant relation with CCI, with reasonably high coefficient of determination ( $R^2 = 0.64$ ) and significant  $P$ -value ( $P < 0.001$ ). Significant relation of chlorophyll content with  $CI_g$  could reflect ability of the spectral indices from *in-situ* hyperspectral measurements to estimate chlorophyll content in crop canopies. The chlorophyll content thus estimated could improve GPP prediction by LUE models by avoiding the bias involved in determining green LAI.

### Relationship between $P_N$ and VI and environmental variables

Photosynthesis in plant canopies is greatly influenced by both canopy structural attributes (e.g. LAI, phenology)

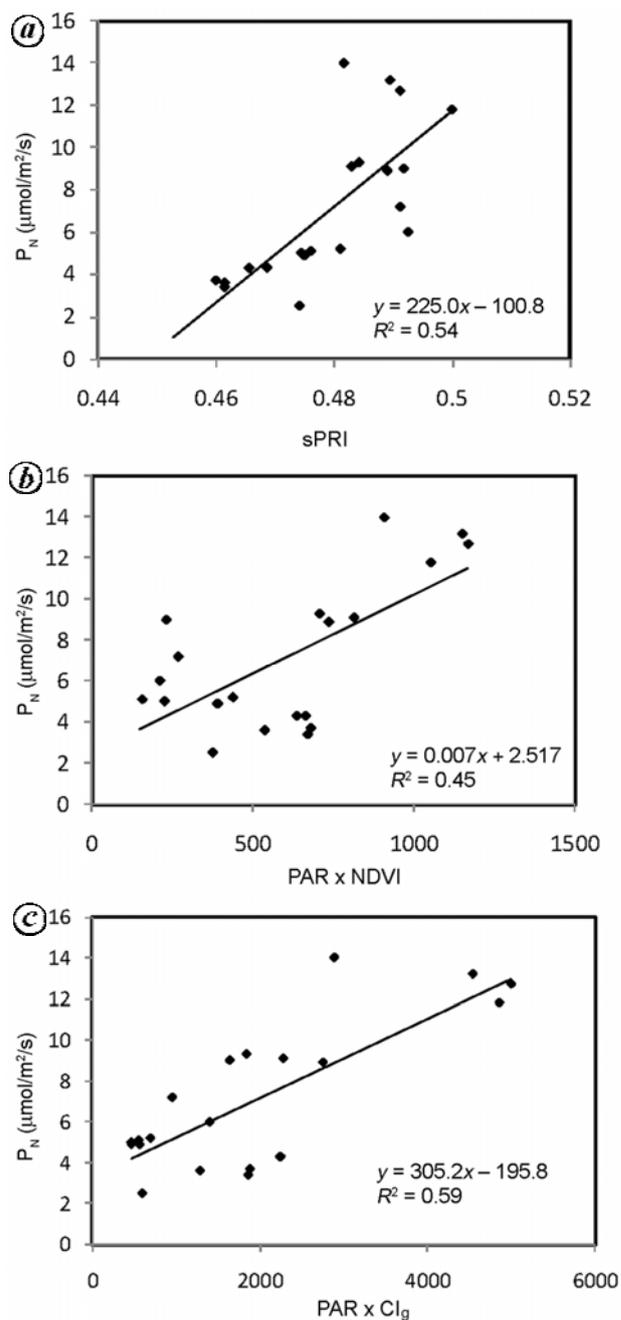
**Table 2.** Average values of environmental variables at two growth stages of rice in the study area

Characters	Mean $\pm$ SE	
	29.09.11 (Anthesis stage)	14.10.11 (Senescence stage)
Net photosynthesis rate ( $P_N$ ) ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	$12.1 \pm 1.07$	$4.04 \pm 0.27$
Chlorophyll concentration index (CCI)	$24.95 \pm 2.81$	$23.02 \pm 3.70$
Air temperature ( $^{\circ}\text{C}$ )	$39.2 \pm 0.44$	$39.56 \pm 0.48$
Leaf temperature ( $^{\circ}\text{C}$ )	$38.76 \pm 1.01$	$39.44 \pm 0.69$
Photosynthetically active radiation (PAR; $\mu\text{mol}/\text{m}^2/\text{s}$ )	$1199.25 \pm 65.82$	$878 \pm 57.38$



**Figure 2.** Relationship between *in-situ* measured chlorophyll concentration index (CCI) and spectrally derived green chlorophyll index ( $CI_g$ ) using hyperspectral measurement.

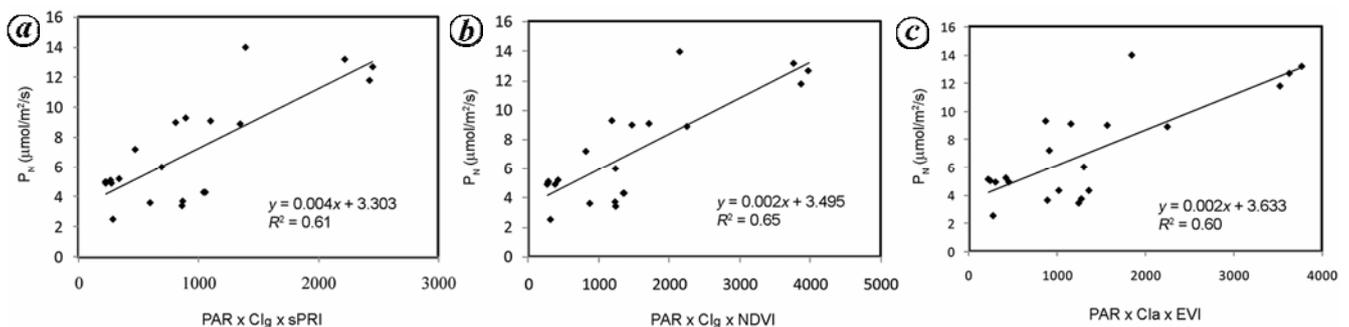
and environmental drivers (e.g. PAR, temperature, vapour pressure deficit, etc.). Hyperspectral indices have been recently used in estimating gross or net photosynthesis because they are the best proxy for canopy variables (e.g. LAI, chlorophyll content and  $fAPAR$ ) governing this process. In this study, we explore potential of both difference-based (EVI, NDVI) and chlorophyll-related ( $CI_g$ , sPRI) indices in estimating  $P_N$ . Least-square regression reveals that either EVI or NDVI had a poor relationship with  $P_N$ . However, among all the spectral indices, sPRI explained the highest variance in net photosynthesis ( $R^2 = 0.543$ ; Figure 3). Strong and significant relationship of sPRI with  $P_N$  could be attributed to the characteristic nature of sPRI as an indicator of photosynthetic efficiency or LUE<sup>30,31</sup>. Other indices are mainly a surrogate of green LAI/ $fAPAR$ , which is more expressive of the photosynthetic apparatus. Overall spectral indices performed poorly because of several reasons. A simple model based on VI alone does not address all the factors which influence the estimation of GPP. This simple model does not provide any means for assessing the timing of the photosynthetic dormant period or for tracing the seasonal  $P_N$  fluctuations subject to drought conditions besides the short-term deviation in GPP due to environmental stresses (e.g. temperature, humidity, soil moisture) as these short-term stresses do not affect crop greenness<sup>32</sup>.



**Figure 3.** Relationship between *in-situ* measured photosynthesis rate ( $P_N$ ) against hyperspectral-derived indices: (a) Scaled photochemical reflectance index (sPRI), (b)  $PAR \times NDVI$  (normalized difference vegetation index) and (c)  $PAR \times CI_g$ .

**Table 3.** Regression results between  $P_N$  and VIs, environmental variables and VI-based combinations with environmental variable

Parameters and combinations	$R^2$	$t$	$P$	$N$	$A$ (intercept)	$B$ (slope)	Standard error of estimate
EVI	0.09	1.37	0.1800	21	2.80	6.33	3.47
NDVI	0.40	3.59	0.0019	21	-6.33	19.12	2.81
sPRI	0.54	4.75	0.0001	21	-100.82	225.03	2.46
CI <sub>g</sub>	0.18	2.07	0.0522	21	4.52	1.02	3.28
PAR	0.26	2.59	0.0177	21	3.21	0.00	3.12
CCI	0.22	1.99	0.0657	18	3.89	0.193	3.13
PAR × EVI	0.38	3.43	0.0027	21	2.85	0.01	2.85
PAR × NDVI	0.45	3.94	0.0008	21	2.51	0.007671	2.69
PAR × sPRI	0.30	2.88	0.0095	21	2.97	0.010237	3.03
PAR × CCI	0.56	4.22	0.0008	18	4.88	0.000174	2.36
PAR × CI <sub>g</sub>	0.59	5.23	0.0000	21	3.26	0.0019	2.32
PAR × EVI × EVI	0.42	3.69	0.0015	21	3.31	0.01	2.77
PAR × EVI × sPRI	0.42	3.73	0.0014	21	2.74	0.02	2.76
PAR × EVI × NDVI	0.52	4.51	0.0002	21	2.79	0.010736	2.52
PAR × CCI × EVI	0.61	4.72	0.0003	18	4.41	0.000271	1.99
PAR × CCI × NDVI	0.62	4.79	0.0002	18	4.84	0.00023	2.18
PAR × CCI × sPRI	0.57	4.37	0.0006	18	4.92	0.00024	2.20
PAR × NDVI × sPRI	0.49	4.30	0.0004	21	2.52	0.02	2.59
PAR × CI <sub>g</sub> × sPRI	0.61	5.43	0.0000	21	3.30	0.00396	2.27
PAR × CI <sub>g</sub> × NDVI	0.65	5.99	0.0000	21	3.49	0.00244	2.14
PAR × CI <sub>g</sub> × EVI	0.60	5.38	0.0000	21	3.63	0.002510	2.29

**Figure 4.** Relationship between *in-situ* measured  $P_N$  and hyperspectral-derived VI-based combinations: (a)  $PAR \times CI_g \times sPRI$ ; (b)  $PAR \times CI_g \times NDVI$  and (c)  $PAR \times CI_g \times EVI$ .

### Relationship between $P_N$ and VI based combinations

Temporal behaviour of photosynthesis mainly responds to changes in phenological and physiological status, which is closely related to leaf chlorophyll content and thus to spectral indices (e.g. sPRI, NDVI). The VI-based model does not account for high frequency variation caused by PAR. In order to improve the estimation of  $P_N$  more accurately, a product of VI and PAR was considered for evaluation (Table 3). It was found that product of VI × PAR did not lead to noticeable improvement of variance explained in  $P_N$  over VI alone. However, among all combinations of VI × PAR, a product of  $CI_g$  and PAR yielded a relatively higher determination coefficient ( $R^2 = 0.59$ ) with significant probability ( $P$ -value = 0.0008). Improvement in regression fit of  $P_N$  with  $CI_g \times PAR$  could reflect better representation of photo-

synthetic potential (i.e. APAR) by  $CI_g$ , which acts as proxy of  $fAPAR$  and is directly related to chlorophyll content (Figure 4).

Net photosynthesis is an indicator of stored biomass of the ecosystem which depends on the photosynthetic efficiency of crop species, the canopy structure and the illumination conditions. Therefore, we used a form of the LUE model that considers  $P_N$  as a product of VI, VI and PAR and is similar to the Monteith logic. We have explored the potential of the following model for  $P_N$  estimation by using different VIs.

$$GPP \propto VI \times VI \times PAR.$$

$P_N$  was successfully estimated with the above-mentioned model and moderate  $R^2$  was observed for relationships comprising chlorophyll (Table 3). The  $R^2$  of  $P_N$  estima-

tion was improved compared to the results obtained using a single index. PAR  $\times$  CCI  $\times$  NDVI, PAR  $\times$  CCI  $\times$  EVI and PAR  $\times$  CCI  $\times$  sPRI have demonstrated the suitability of the models for  $P_N$  estimation with  $R^2$  of 0.62, 0.61 and 0.57 respectively. The use of  $CI_g$ , a chlorophyll index, in the VI  $\times$  VI  $\times$  PAR model has also shown better  $R^2$  (0.60, 0.65 and 0.60) and represented its importance in  $P_N$  assessment. The reason is that chlorophyll can be a reliable proxy of LUE and VI is a good indicator of LAI (a proxy of  $fAPAR$ )<sup>17,20,23,24,33,34</sup>. PAR absorbed by photosynthetic pigments, especially chlorophyll content, enabled photosynthetic processes, whereas PAR absorbed by non-photosynthetic components such as branches, stems and litter will contribute little towards  $CO_2$  fixation<sup>35</sup>. Therefore, it can be concluded that a single index is insufficient to address all components whereas chlorophyll based and other vegetation indices as proxy of LUE and  $fAPAR$  respectively, describe all the components of Monteith logic for the enhanced  $P_N$  estimation.

## Conclusions

We have studied parametric relationships between  $P_N$  estimated by portable photosynthesis system and spectral indices from *in-situ* hyperspectral measurements over rice in a subtropical environment. We have attempted to test and justify performance of difference-based and chlorophyll-related indices in estimating net photosynthesis. The results reveal that green chlorophyll index based on NIR (800 nm) and green (550 nm) wavelength were strongly related to CCI as a measure of chlorophyll content. Performance of the model formulations improved with the inclusion of VIs in combination with PAR, because such formulation follows the Monteith logic of  $P_N$  estimation in an ecosystem ( $P_N = LUE \times fAPAR \times PAR$ ). The model involving either green chlorophyll index or direct measure of chlorophyll (CCI) in Monteith logic was found to be superior in estimating photosynthesis in rice. Results provide insights into improvement in the modelling of photosynthesis over an ecosystem by using chlorophyll content-related VIs from future space-borne hyperspectral sensors.

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