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Abstract. Photosynthetic pigment concentration has strong relationship with nitrogen (N) concentration which is an essential element of plant growth and plays an important role in estimating the net primary productivity (NPP) in terrestrial ecosystem research and precision agriculture (PA). In this study, hyperspectral reflectance and pigment concentration of the upper three leaves of rice crop (*Oryza sativa* L.) with five N fertilization rates were measured in the laboratory. The results showed that there was no significant difference between the leaf hyperspectral reflectance and pigment concentrations in the visible and near-infrared (NIR) spectral regions at each leaf position. But in the shortwave infrared (SWIR) region, the difference of each two of the three leaf positions was obviously significant at level 0.05. The integrated hyperspectral index $MCARI/OSAVI_{[670,800]}$ had been proved to be better linear related with leaf pigment concentrations at different leave position. The result demonstrated that $MCARI/OSAVI_{[670,800]}$ was a reliable and stable hyperspectral index for estimating pigment concentration at leaf level..

Keywords: Hyperspectral reflectance; Nitrogen fertilization rate; Pigment concentration; Rice

1 Introduction

Net primary productivity (NPP) is a function of an efficiency coefficient defining the carbon dioxide (CO_2) fixed in short-lived (foliage and fine roots) and long-lived (wood) tissue per unit area and time[1-2], which plays a vital role in essential materials and suitable environments for human society[3-4]. The precise estimation of NPP over a continental and global scale would facilitate an improved interpretation of dynamic physiological processes within terrestrial ecosystems research and precise agriculture (PA) management, and is also crucial for the research on the relationship between global climate change and carbon cycling processes[5-6].

NPP has been shown to be related to the fraction of absorbed photosynthetically active radiation (f_{apar}) which is a function of the sum of the concentrations, and more precisely the molar extinctions, of chlorophyll a (Chl *a* hereafter), chlorophyll b (Chl *b*), and the carotenoids (Cars). In addition, leaf characteristics, especially nitrogen (N) content, and specific leaf area affect NPP directly by constraining photosynthesis and transpiration rates[7-9]. Moreover, N is an essential element for plant growth and is frequently the major limit nutrient in most agricultural soils. Excessive N fertilizer may move into surface water and groundwater and accelerate eutrophication of lakes and streams[10]. Farmers must balance the competing goals of supplying enough N to their crops in PA management while minimizing the loss of N to the environment, which represents both a threat to water quality and an economic loss. Since N is the component element of chlorophyll molecules, there is a close link between leaf chlorophyll content and leaf N content[11]. Thus remote sensing techniques have the potential to evaluate the chlorophyll variability over large fields quickly[12].

Developments in hyperspectral remote sensing made it possible to estimate N and NPP variability through quantifying individual photosynthetic pigments within vegetation. Spectral reflectance measurements of corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) canopies have been used to detect characteristic of different N status of crop and provide reliable information for developing variable-rate fertilizer N application technique[13-15]. The variety of N treatments resulted in the differences in leaf chlorophyll content, leaf area index (LAI), biomass, and foliage cover which contributed to the differences in spectral reflectance at the leaf and canopy scales. The relatively subtle differences in leaf and canopy reflectance associated with changes in leaf chlorophyll are often confounded with major changes in plant growth and development due to N treatments[12].

Vegetation indices (VIs) have been developed as an attempt to reduce spectral effects caused by external factors such as the atmosphere and the soil background[16]. Peñuelas et al.[17] have advocated a shift towards narrow-band VIs for estimating the absolute and relative concentrations of Chl *a*, Chl *b*, and Cars in plant leaves. Zarco-Tejada et al.[18] performed a study of VIs for chlorophyll estimation in open-canopy forest from leaf level to the canopy through *SAIL and Kuusk* canopy reflectance model and demonstrated that nominal canopy reflectance model parameters appear to be sufficient to allow accurate application of the optical index/bioindicator algorithm to airborne data. Research by Wu et al.[19] suggested that the integrated VIs, namely, TCARI/OSAVI $_{[705,750]}$ and MCARI/OSAVI$_{[705,750]}$, were most appropriate for chlorophyll estimation with high correlation coefficients R^2 of 0.881 and 0.941, respectively, because more disturbances such as shadow, soil reflectance and nonphotosynthetic materials were taken into account.

Therefore, this study aims at examining the performance of integrated hyperspectra indices MCARI/OSAVI$_{[670,800]}$ developed by Rondeaux et al.[20] and Daughtry et al. [12] in photosynthetic pigment concentration estimation within the rice leaf. Meanwhile, the feedback response of leaf spectral characteristics to leaf pigment concentration due to N treatments will be investigated.

2 Materials and Methods

2.1 Field Experiment

The field experiment was conducted on sandy loam soil from early June to late October in 2008 at the research farm of Xiaoshan Agricultural Science Research Institute (30°20′N latitude, 120°31′E longitude and altitude approximately 6m), Hangzhou, Zhejiang Province, China. The original soil had 13.1g/kg organic C, 5.6mg/kg bicarbonate extractable P, 35.2mg/kg exchangeable K, 1.26g/kg total N and Ph 7.5 (soil: water = 1:1 (w/v)). Three rice (*Oryza sativa* L.) cultivars, namely, Yongyou 8, Zhongzheyou 1, and Zhejiang 22, were employed in this study.

A random block design was employed with the three rice cultivars and five N fertilization rates (0, 75, 180, 285 and 390 kg/ha). Forty percent of the N fertilizer (i.e. urea) was fertilized during the pre-transplanting period, 30% at the tillering stage, and 30% at the initial heading stage. Each treatment was replicated three times. The rice plants were transplanted on July 5th, 2008 and harvested on October 31st, 2008.

2.2 Hyperspectral Reflectance Data Acquisition

One whole rice plant from each plot was collected, placed in a plastic bucket with water, and transported to the laboratory for spectral measurement on August 21st, September 27th, and October 10th, 2008. The first, second and third uppermost leaves were cut from the main stem of rice plant and referred to as L1, L2 and L3, respectively. The hyperspectral reflectance of L1, L2 and L3 was measured with a portable spectrophotometer (Analytical Spectral Devices, Inc., Colorado, CO, USA) in the wavelength range of 350-2500 nm. The spectral resolution of the instrument is 3 nm for the region of 350-1000 nm and 10 nm for the region of 1000-2500 nm.

Rice leaves were positioned on a dark background so that the fiber optic sensor with a 25° instantaneous field of view (IFOV) vertically pointed to centre of the leaf surface with about 3.5 cm height, equivalent to 1.9 cm^2 observed area. An incidence angle of 45° was maintained at a standard distance of 50 cm throughout the study in a closed chamber with a halogen lamp (50 Watt). A sampling spectrum was consisted of ten readings for each leaf. The average spectra derived from the sampling spectra represented each leaf using ViewSpec Pro (version 5.6.10).

2.3 Foliar Pigment Concentration Acquisition

After the hyperspectral measurements, a leaf disk with a weight of 0.1 g was cut from each leaf for pigment analysis. Each leaf disk was crushed, and dipped in 20 ml solution (acetone: ethanol: distilled water = 4.5:4.5:1) for 24 hours in the dark environment to extract pigment. The optical density (OD) of the extraction solution was measured at 440 nm, 645 nm, and 663 nm by spectrophotometer (Shimadzu UV 2550, Tokyo, Japan). Pigment consists of Chl *a*, Chl *b*, and Car in the study. The formula of pigment concentration (mg/g) was referenced the literature of Tang et al.[21].

2.4 Data Analysis

2.4.1 Data Preprocessing

The hyperspectral reflectance was smoothed with a five step moving average to suppress instrumental and environmental noise before these data were further analyzed[22]. Then, the raw hyperspectral reflectance less than 400 nm and more than 2400 nm were ineffective due to severe instrument and system noise[23].

2.4.2 Statistical Analysis

Analysis of variance (ANOVA) is useful for assessing what proportions of the variations in a dependent variable can be accounted for by one or more independent variables[20, 24]. The hyperspectral reflectance (Table 1) and pigment concentration (Table 2) were expressed as mean and standard deviation (SD) for the remote sensing and biophysical data and were analyzed by ANOVA, followed by Duncan's multiple-range test when appropriate. Differences between groups were considered significant when $p<0.05$. Pearson's linear regression coupled to ANOVA was used to verify the effects of pigment concentration on hyperspectral reflectance and integrated hyperspectral VIs. Values of the determination coefficients were obtained by the linear regression analysis (Fig.3). ANOVA was then performed with SPSS software (Statistical Package for the Social Science, version 16.0.0).

2.4.3 Integrated Hyperspectral index

The chlorophyll absorption ration index (CARI) developed by Kim[25] could minimize the effects of nonphotosynthetically materials on spectral estimates of absorbed photosynthetically active radiation (PAR). Daughtry et al. [12] simplified the calculation equation of CARI to obtain the modified CARI (MCARI). MCARI is the depth of chlorophyll absorption at 670 nm relative to the reflectance at 550 nm and 700 nm and is defined as the following equation:

$$MCARI_{[670,700]}=[(R_{700}-R_{670})-0.2(R_{700}-R_{550})]\frac{R_{700}}{R_{670}} \qquad (1)$$

Rondeaux et al.[20] concluded that the optimized soil-adjusted vegetation index (OSAVI) could reduce the sensitivity of object reflectance to the underlying soil. OSAVI is defined as follows:

$$OSAVI_{[670,800]}=\frac{(1+0.16)(R_{800}-R_{670})}{(R_{800}+R_{670}+0.16)} \qquad (2)$$

Therefore, the integrated form of MCAI and OSAVI could be defined as

$$\frac{\text{MCARI}}{\text{OSAVI}}[670,800] = \frac{[(R_{700}\text{-}R_{670})\text{-}0.2(R_{700}\text{-}R_{550})](R_{700}/R_{670})}{(1+0.16)(R_{800}\text{-}R_{670})/(R_{800}+R_{670}+0.16)} \quad (3)$$

Wu et al.[19] proved that MCARI/OSAVI is one of the most appropriated integrated hyperspectral indices for chlorophyll estimation because more disturbances such as shadow, soil reflectance and nonphotosynthetic materials are taken into account.

3 Results

3.1 Leaf Hyperspectral Reflectance

The mean (n = 45) hyperspectra reflectance of L1, L2, and L3 of rice crops collected on three sampling dates for three cultivators at five N fertilization rates were shown in Fig.1. They showed very little detail information in the full wavelength range (400-2400 nm), especially in the visible region.

To get more specific information on the difference between different leaf positions in leaf hyperspectral reflectance due to changes of leaf pigment concentration, the hyperspectral reflectance as waveband means was calculated to simulate the Enhanced Thematic Mapper Plus (ETM+) of Landsat-7 (Table 1). ANOVA followed by Duncan's test with 0.05 of alpha was used to verify the effects of pigment concentration at different leaf positions on hyperspectral reflectance (Table 1 and 2).

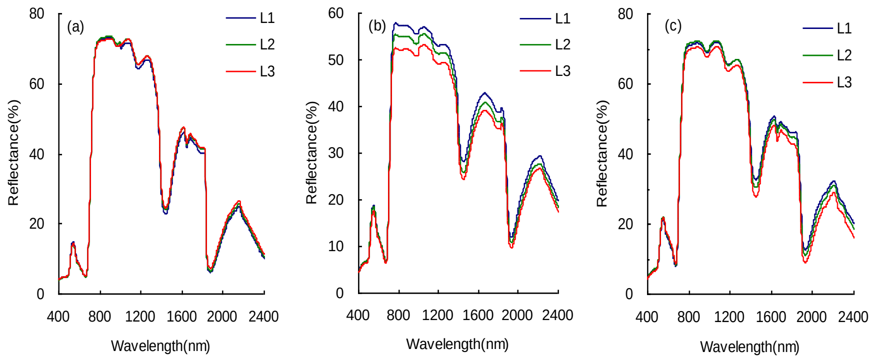


Fig.1 Mean hyperspectral reflectance of rice crop at the first, second and third uppermost leaf (L1, L2 and L3) collected on August 21st (a), September 27th (b) and October 10th (c), 2008.

Table 1 Average hyperspectral reflectance of rice crop at the first, second and third uppermost leaves (L1, L2 and L3) at six wavelength intervals denoting the Enhanced Thematic Mapper Plus (ETM+) of Landsat-7 (Unit :%)*

Specta range (nm)	August 21st, 2008			September 27th, 2008			October 10th, 2008		
	L1	L2	L3	L1	L2	L3	L1	L2	L3
Blue(450-515)	6.0a	5.8a	6.0a	7.5a	7.5a	7.1a	8.0a	8.2a	7.7a
Green(525-605)	11.8a	11.2a	11.5a	16.0a	15.8a	14.9a	18.7a	19.3a	19.3a
Red(630-690)	6.4a	6.1a	6.5a	8.1a	8.1a	7.7a	10.2a	10.6a	11.1a
NIR(775-900)	72.7a	73.1a	72.4a	57.4a	55.1b	52.2c	71.2a	71.8a	70.0a
$SWIR_1$(1550-1750)	43.6b	44.7a	45.0a	41.4a	39.3a,b	37.7b	48.6a	47.7a,b	45.9b
$SWIR_2$(2090-2350)	19.9b	20.5a	21.4a	26.9a	25.5a,b	24.4b	28.4a	27.1a	24.8b

* Means followed by the same letter in each row are not significantly different from each other by Duncan test at alpha = 0.05.

Table 2 Statistical description of pigment concentration for the first, second and third uppermost leaves (L1, L2 and L3)

Date	Pigment	Chlorophyll a			Chlorophyll b			Carotenoids		
	Position	L1	L2	L3	L1	L2	L3	L1	L2	L3
08/21	Mean	1.97a	2.12a	2.07a	0.66b	0.71a,b	0.75a	0.74b	0.81a	0.80a
	SD	0.32	0.33	0.45	0.12	0.14	0.16	0.12	0.12	0.13
09/27	Mean	2.40a	2.19a	2.11a	0.82a	0.80a	0.81a	0.91a	0.81a	0.81a
	SD	0.82	0.89	0.80	0.31	0.36	0.35	0.27	0.26	0.26
10/10	Mean	1.63a	1.39a	1.02b	0.53a	0.48a	0.37b	0.75a	0.65a	0.50b
	SD	0.76	0.74	0.55	0.28	0.27	0.22	0.27	0.28	0.21

*Means followed by the same letter in each row are not significantly different from each other by Duncan test at alpha = 0.05.

Photosynthetic pigments control the hyperspectral reflectance and transmittance in the visible region. Because there was almost no significant difference among L1, L2 and L3 in concentration of Chl *a*, Chl *b* and Cars (Table 2), the hyperspectral reflectance had no corresponding significant difference in the visible blue-green (450-515nm), green (525-605nm), red (630-690nm), near-infrared (NIR, 775-900nm) spectral regions except the samples collected on September 27th, 2008 as shown in Table 1.

In the shortwave infrared spectral region ($SWIR_1$:1550-1750nm; $SWIR_2$:2090-2350nm), the differences between L1 and L2, and between L2 and L3 were significant only for the latter two sampling dates. Comparing with L1 collected on September 27th, 2008, the hyperspectral reflectance of L2 decreased 5.1% and 5.2% in $SWIR_1$ and $SWIR_2$ regions, respectively; and comparing with L2, the hyperspectral reflectance of L3 decreased 4.1% and 5.3% in $SWIR_1$ and $SWIR_2$ regions, respectively(Fig.1 *b* and Table 1). Same phenomena occurred for the third sampling date (Fig.1 *c* and Table 1). But the very reverse changes in hyperspectral reflectance appeared for the first sampling date (Fig.1 *a* and Table 1).

3.2 Relationship Between Hyperspectral Reflectance and Leaf Pigment Concentration

The correlation between the hyperspectral reflectance and the leaf pigment concentrations was investigated to interpret the changes of spectral characteristics through the full wavelength range (400-2400 nm) (Fig.2).

As shown in Fig.2(*a*), the hyperspectral reflectance in the green light region had greatest negative correlation with leaf pigment concentrations. The correlation coefficients were 0.735, 0.660 and 0.708 for Chl a, Chl b and Cars, respectively; and the corresponding wavebands of the former pigment located at 560nm, the latter two pigments at 545nm. In the red light region, the highest and lowest coefficients of hyperspectral reflectance with leaf pigment concentrations appeared near 667nm and 694nm wavebands, respectively. The inflexion points of correlation coefficient in the NIR region came forth near 760nm. The highest and lowest coefficients in SWIR region rose near 1458nm and 1650nm, but the former waveband would in the vapor absorption region if the hyperspectral reflectance was measured at the canopy level from the airborne or spaceborne platforms. Although the growth stages were different (Fig.2 *a*-*d*), the sensitive wavebands of hyperspectral reflectance to leaf pigment concentrations always occurred near 550nm, 670nm, 700nm and 1450-1460nm in the green, red, NIR and SWIR regions, respectively.

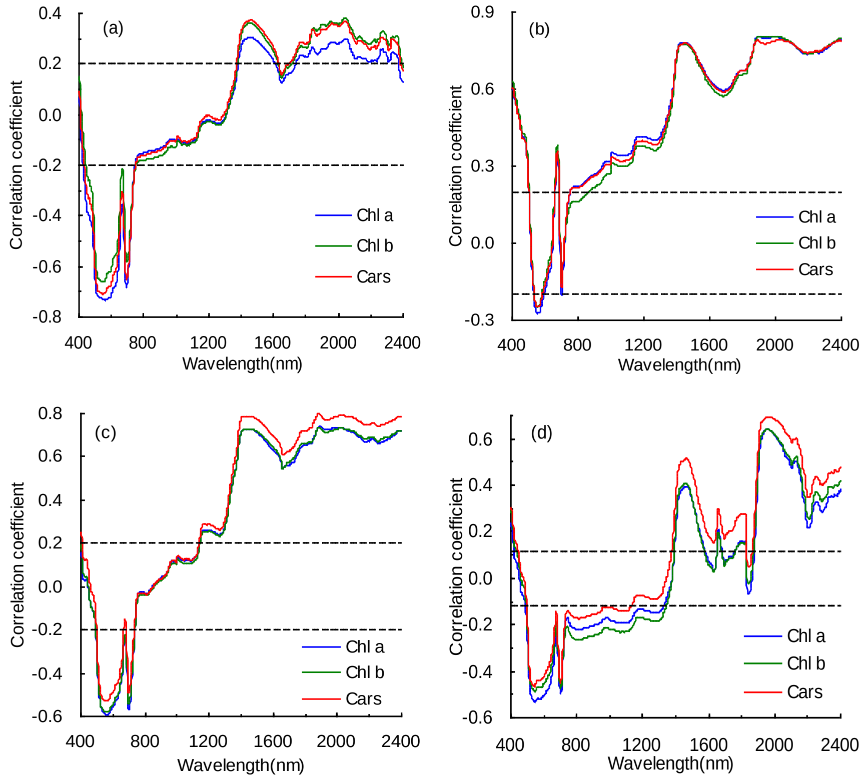
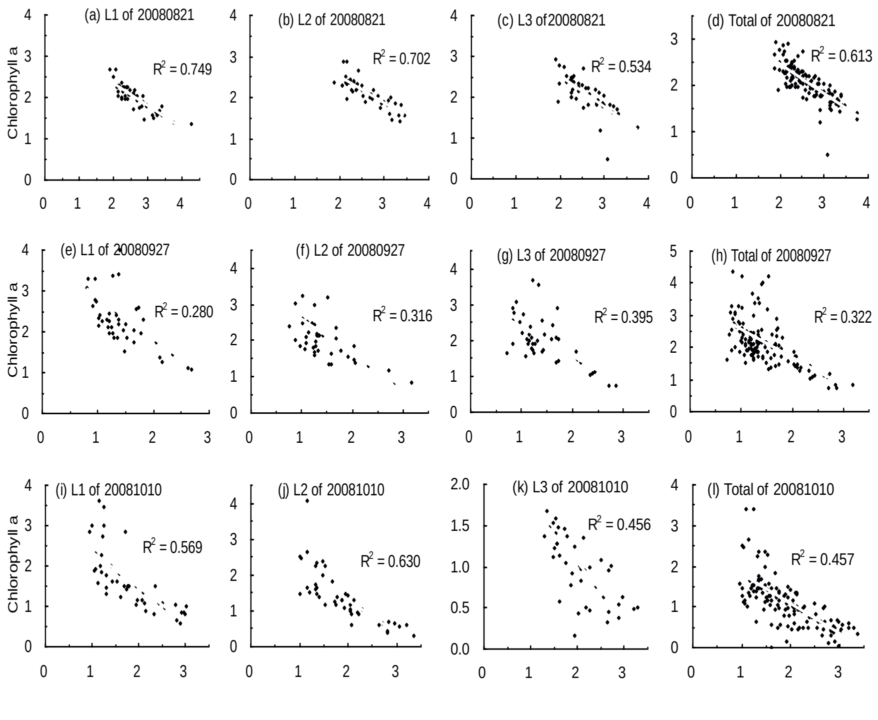


Fig.2 Correlograms of hyperspectral reflectance to leaf pigment concentration collected on August 21st (a)，September 27th (b) and October 10th (c), 2008, and all the three sampling dates (d). The dashed line denoted obviously significant level at p<0.001.

3.3 Regression Between Pigment Concentration and Integrated Hyperspectral Index

As analyzed above, the sensitive wavebands were consistent with the applied wavebands of integrated hyperspectral index ($MCARI/OSAVI_{[670,800]}$). $MCARI/OSAVI_{[670,800]}$ had better linear relationship with leaf pigment concentrations (Fig.3). As shown in Fig.3 (*a*) to (*l*), the correlation between $MCARI/OSAVI_{[670,800]}$ and leaf pigment concentrations were obviously significant ($p<0.05$) in any leaf positions and total samples. Strong correlation existed for all the data collected in the three sampling dates (Fig.3 *m-p*). $MCARI/OSAVI_{[670,800]}$ were most appropriate for Chl *a* estimation with high determination coefficient (R^2) of 0.749 for L1 collected on August 21st, 2008 (Fig.3 *a*). It was worst appropriate for all the data collected in the three sampling dates without regard to leaf positions (Fig.3 *p*). The determination coefficients of total samples for any sampling dates (Fig.3 *d*, *h*, *l* and *p*) were always lower than those of each leaf position (Fig.3 *a-c*, *e-g*, *i-k* and *m-o*).



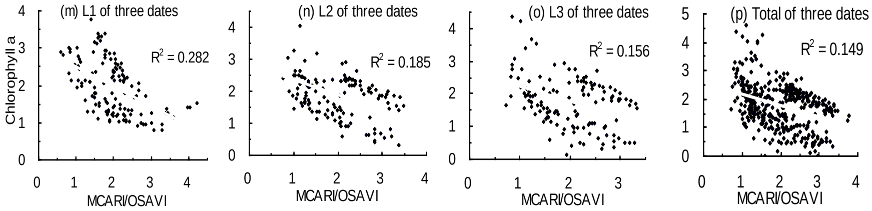


Fig.3 Linear relationship between foliar chlorophyll a concentration of rice crop and the integrated index (MCARI/OSAVI$_{[670,800\ ]}$)

4 Discussion and Conclusion

In the present study, the hyperspectral reflectance were found that there was no significant difference (n=45 in each group) in the visible blue (450-515nm), green (525-605nm), red (630-690nm), near-infrared (NIR, 775-900nm) spectral regions for difference leaf positions (Fig.1, Talbe1) because the difference in concentrations of photosynthetic pigments Chl *a*, Chl *b* and Cars, which absorb the visible spectrum by electronic transitions among L1, L2 and L3 was almost not significant (Table 2). In SWIR regions, however, the difference was obviously significant (Duncan test at alpha = 0.05) by twos of all the three leaf positions (Table 1). The leaf hyperspectral reflectance in SWIR regions decresed gradationally from the top to below in the two out of three sampling dates (Fig.1, Table 1). Such results were similar to others reports by Zhao et al.[3] who concluded that the hyperspectral reflectance of maize would change in different spectral regions due to N treatments.

The correlation analysis of hyperspectral reflectance indicated that the most sensitive wavebands mainly located at near 550nm, 670nm, 700nm, 1460nm, and 1650nm. These wavebands always presented the greatest correlation coefficient with significant difference (p<0.05) even very obviously significant difference (p<0.001). The result in this study was incompletely with the current research which suggested that the most sensitive wavebands distributed in the green (525-605nm) and yellow (605-650nm) regions, and the worst in SWIR region[26]. The waveband at 1460nm was efficient to estimate the absolute and relative concentration of photosynthetic pigments and other biophysical or biochemical parameters at leaf level in the laboratory condition, but it's inefficient at the canopy level from the near ground, airborne and spaceborne platforms because of the presence of water vapor[12,27].

The integrated hyperspectral index MCARI/OSAVI$_{[670,800]}$ had the better linearity with leaf pigment concentrations(Fig.3). Perhaps because the component wavebands were derived from the aforementioned sensitive wavebands (Equ. (3)). As shown in Fig.3 (*a*) to (*l*), The regression analysis denoted that MCARI/OSAVI $_{[670,800]}$ had a great relationship with leaf pigment concentrations in any leaf positions and total samples. To take into account the leaf positions, MCARI/OSAVI$_{[670,800]}$ was a reliable hyperspectral index for estimating the pigment concentration.

However, there are more external factors including canopy structure, LAI, plant cover density, soil types, soil moisture, sunlight illumination, cloudy and plant shadow, and so forth, which coexist in the field[23], and bring lots of noise in remote sensing application. Therefore, it will be necessary to make multiangular measurement to acquire more detailed information on plant structure than vertical measurement, and then the hyperspectral VIs will obtain high accuracy for estimating the concentration of photosynthetic pigments and other agricultural parameters.

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