

Enhanced broadband greenness in assessing Chlorophyll a and b, Carotenoid, and Nitrogen in Robusta coffee plantations using a digital camera

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Abstract Direct-leaves measurement of spectral indices using a digital camera with a portable small chamber and custom illumination is used to take images of 600 leaves from 40 coffee plants. In this research, several vegetation indices (VIs) are developed and evaluated. Through a series of experiments, Chlorophyll a and b, Carotenoids, and Nitrogen critical level of Robusta coffee plants are analyzed and evaluated using selected VIs obtained from spectra of different tools like Spectrometer, digital camera, and SPAD-502 Chlorophyll meter. The actual Nitrogen critical level was determined using Kjeldahl laboratory test. Beside Hue, the newly proposed VIs could significantly improve the correlation in estimating photosynthetic pigments (Chlorophyll a and b, Carotenoids) and Nitrogen critical level of Robusta coffee plant. Finally, consumer-grade digital camera with custom chamber is shown to be used for rapid and accurate in situ estimation of Chlorophyll a and b, Carotenoids, and Nitrogen critical level of Robusta coffee plant from direct-leaves measurement.

Keywords Broadband greenness · Photosynthesis pigments · Nitrogen · Direct-leaves measurement · RGB camera · Broadleaf plant

Introduction

Most of the smallholders coffee plantations in developing countries are located in tropical region. In Indonesia, coffee plantation sprawls over more than 1.3 million ha of which 96% plantations belong to smallholders and the remaining 4% to private and government-

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owned estates (MOA 2014). Changing climate could adversely affect coffee plantation and yields. For instance, high intensity rainfall could cause high humidity, eventually resulting in increased emergence of various diseases in the Robusta coffee plants whereas, during dry season, the plants were subjected to accelerated evapotranspiration. In these conditions, farmers face the challenge of estimating the actual nutrients demand of coffee plants due to uncontrolled/unaccounted for plants growth and photosynthesis process.

In measuring availability of Nitrogen nutrient in plant, the traditional destructive method of plant tissue analysis is the most reliable, but this method is unacceptably expensive, and the laboratory results cannot be immediately known by farmers to determine fertilizer requirements. In this destructive method, considering Nitrogen fluctuations in soil as well in plants, the leaf tissue test is more relevant to the plants than soil test (Edward et al. 2005). Data from leaf tissue analysis of Nitrogen status may be more reliable than data from Nitrogen soil analysis because roots explore more soil than is normally sampled by coring (Li et al. 2010). These destructive tests popularly follow Kjeldahl laboratory procedures; this method is considered reliable but is time consuming and expensive. Moreover, the farmers are not technically competent to follow scientific procedures. In addition, over application of Nitrogen is the common phenomenon at farmers/smallholders level. This over application of Nitrogen fertilizer in coffee plants is the key cause of low Nitrogen efficiency, environmental pollutions, decreased yield and thereby decreased farmer's income. Moreover, Altieri and Nicholls (2003) showed that the excess Nitrogen fertilizer to the plant reduces resistance to insect pests.

Precision agriculture could help in Nitrogen management to achieve maximum production per unit of the nutrient applied, which depends on the Nitrogen demand of the plants during its various growth stages. Nitrogen critical values in plants could be estimated by visual identification, which reduces time and expenses when compared to the conventional destructive methods (i.e. a laboratory test of leaves and soil). There are several non-destructive methods available to determine the Nitrogen in plants to provide the fertilizer recommendations, mostly based on aerial and ground-based remote sensing. The use of multispectral sensors from aerial platforms such as satellite, airborne, and unmanned aerial vehicles (UAV) have potential in assessing Nitrogen over a wide region. However, limitations of these technologies for commercial use at individual farmer's field or household are: (a) high cost of obtaining images from airborne platform, (b) risky use of UAV in plantations that have tree shade, (c) hilly terrain of the plantation area, (d) inability of satellite to identify single plant or narrow plantation areas, and (e) error induced by weather conditions in satellite images. Furthermore, in the plantation areas those are covered by trees shade, inaccuracy of spectral information increases, resulting in inappropriate Nitrogen fertilizer recommendations. Especially for Robusta coffee plants that are grown in the lowlands area, shade trees are needed to help forming a microclimate in accordance with optimal growing condition (ICCRI 1999). On one hand, the shade trees are essential in coffee plantation; they tend to limit the use of airborne image acquisition.

Another option of non-destructive methods for plantation areas is the ground-based remote sensing. The active sensors like Green Seekers[®], Crop Circle[®], and passive sensors like spectrometer, NIR camera (Widjaja Putra and Soni 2017) and standard RGB camera (Anderson et al. 2016) could be used for this purpose. Today, cameras based on CCD/CMOS sensor are widely used (Chen et al. 2015), but their use for above-canopy measurement is still limited and less-explored due to uncertainty in the background light intensity. Variations in illumination, crop age, and crop height are the major factors challenging field measurement in plantations. Nevertheless, compared with destructive methods, non-destructive methods could reduce time and costs of estimation and enabling

near-real-time management decisions. SPAD-502 Chlorophyll meter has a potential as non-destructive tool through indirect estimation of Nitrogen; the Nitrogen contents of leaves are highly correlated with the photosynthetic pigments like Chlorophyll a and b, and Carotenoids (Netto et al. 2005). These commercial tools like SPAD-502 Chlorophyll meter for direct-leaves measurement including Green Seeker[®] and Crop Circle[®] for canopy measurement are still expensive for the farmers/smallholders in the developing countries.

To fill these technology and economic gaps for the farmers/smallholders, we explore the use of standard digital camera and enhance the red (R), green (G) and blue (B) bands for direct-leaves measurement while keeping its cost within the affordable reach of the farmers/smallholders. The reflectance values of R and G bands are shown to be used as alternative bands of near-infrared (NIR) and R or red-edge (RE) bands in measuring vegetation indices (VIs), including the leaves greenness, which relate to photosynthetic pigments and Nitrogen.

This paper explains the possibility of estimation by developing new VIs for Chlorophyll a and b, Carotenoids, and Nitrogen level in Robusta coffee plants at different growth stages and field conditions using a digital camera that could be used as low-cost system for direct-leaf measurement.

Materials and methods

Site conditions

Experiments were conducted at the fields managed by Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, Indonesia (8°15'24.6''S 113°36'45.1''E). A total of 600 leaves from purposely grown 40 Robusta coffee plants were examined. The sampled leaves from each plant were stored securely and brought to ICCRI plant tissue laboratory. We chose Robusta coffee plants at different growth stages from 2 to 10 years under varied nutrient regimes (i.e. managed/irrigated and unmanaged/rain fed plantations).

Experiment design

Non-destructive measurements

On each coffee plant, we chose leaf number 2 (in rainfed areas) and leaf number 3 (in irrigated areas) from a plagiotropic branch counting from the apex, until 15 leaves were collected. Only normal leaves, and the leaves not suffered by any pests and diseases, were considered and labeled for further examination (Widjaja Putra and Soni 2017). From each selected coffee plant, the 15 leaves were analyzed using spectrometer, SPAD-502 Chlorophyll meter, and a standard 8-bit digital camera with a small portable chamber. In this set of experiments, we used the digital camera and the spectrometer to develop broadband greenness VIs. These indices are compared with other different VIs for the Robusta coffee plants.

Estimating Chlorophyll a and b, and Carotenoids

Models developed by Netto et al. (2005) were used in estimating Chlorophyll a and b and Carotenoids. These models provided good correlation (R^2 were 0.97, 0.94 and 0.92

respectively) between SPAD-502 value with laboratory results (Eqs. 1–3). The equation of models as follows:

$$\text{Chlorophyll } a = 15.5866 + 1.0338 * \text{SPAD} + 0.0679 * \text{SPAD}^2 \quad (1)$$

$$\text{Chlorophyll } b = 30.1471 - 0.4592 * \text{SPAD} + 0.027 * \text{SPAD}^2 \quad (2)$$

$$\text{Carotenoids} = 42.6458 - 0.8595 * \text{SPAD} + 0.021 * \text{SPAD}^2 \quad (3)$$

Destructive measurements for Nitrogen

After the non-destructive measurements were completed for each coffee plant, we further analyzed the 15 labeled leaves from previous measurement for their Nitrogen. Averages of these individual leaves determine Nitrogen level of the coffee plant. This process is in accordance to ICCRI standards where 15 leaves should be taken from one plant to be analyzed using Kjeldahl laboratory procedure.

Data acquisition

Direct-leaves measurement using spectrometer

Spectrometer measurements on the leaves were performed using Ocean Optic USB2000 + VIS–NIR series of portable spectrometer (Ocean optics, USA). The device provided spectral information between 350 and 1000 nm with resolution of 1.5 nm, enabling to explore the broadband greenness of VIs. This spectrometer used HL-2000 tungsten halogen (Ocean optics, USA) as light source. For measuring the reflectance of the leaf surface, we used optical fiber reflection probes (QR600-7-VIS–NIR, Ocean optics, USA) and a white reflectance standard/spectralon surface (WS-1-SL, Ocean optics, USA) to calibrate the reflectance of the leaves. The calibrated reflectance is expressed as percentage of the reflection of the spectralon. Each spectrum measurement was recorded as an average of 10 scans. The software interface controls the spectrometer and provides graphic and numeric representations of reflectance spectra from the spectrometer.

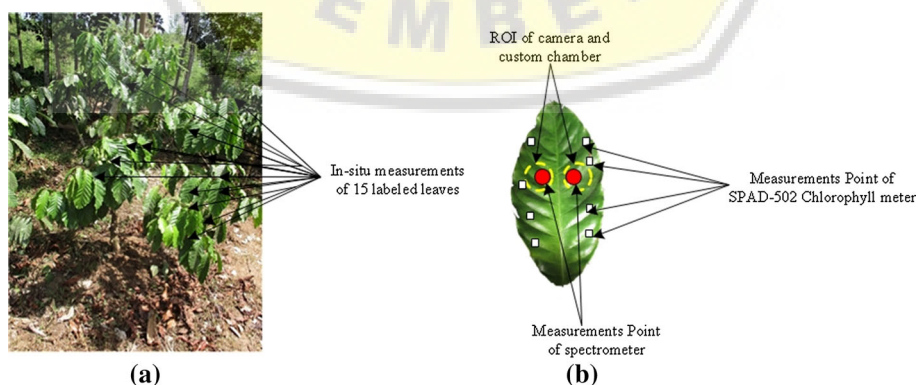


Fig. 1 **a** In-situ measurement of 15 leaves/plant; **b** measurement points and region of interest (ROI) of spectrometer, SPAD-502 Chlorophyll meter, and camera

For each Robusta coffee leaf, the reflectance spectral measurements are taken at two different points and their average was calculated (Fig. 1). Each point measurement was repeated twice to get the average of the spectral values. In total, 2400 reflectance spectral data were collected in this measurement. All leaves were measured individually, by inserting it in probe at 45° angle with probe holder placed perpendicular to the leaf surface.

Direct-leaves measurement using SPAD-502 Chlorophyll meter

SPAD-502 Chlorophyll meter has the proven potential to detect Nitrogen status and could be used as a tool for improving the recommendation of Nitrogen fertilizer (Yuan et al. 2016). All the sampled 600 coffee leaves were measured using the SPAD-502 Chlorophyll meter. The mean of eight SPAD-502 readings is the value of the individual leaf (Fig. 1), and the mean from 15 individual leaf values from each plant was considered the SPAD-502 value for that coffee plant. Care was taken to avoid measuring leaf vein while using SPAD-502 Chlorophyll meter.

Direct-leaves measurement using digital camera

All the sampled 600 leaves were then captured using a 20 mega pixel digital camera that used a charged coupled device (CCD) as image sensor (CANON IXUS 160) with portable small chamber proposed by Widjaja Putra and Soni (2017). We used the digital camera with configuration of auto-focus, automatic exposure time and custom-white balance mode. All leaves were measured individually (captured twice and averaged after extracting the RGB values) (Fig. 1).

Determination of Nitrogen levels

The Kjeldahl method has been widely used for Nitrogen determination especially in plant tissue analysis. The method includes three stages namely the destruction process, distillation and titration. We used this method to estimate the Nitrogen critical levels in 15 selected leaves as representative of the plant Nitrogen.

In this study, after performing non-destructive measurements, all the 600 sampled leaves were analyzed in the laboratory to determine the total Nitrogen. In the Robusta coffee, optimum range of Nitrogen levels of plants are 2.71–3.30% (Willson 1985). Critical levels of Nitrogen in Robusta coffee plants are summarized in Table 1.

Table 1 Critical levels of Nitrogen in Robusta coffee plant using laboratory test (Willson 1985)

Status	Nitrogen (%) ^a
Deficient	<1.80
Subnormal	1.80–2.70
Normal	2.71–3.30
High	>3.30

^a Percentage of Nitrogen critical levels from leaves examination obtained through Kjeldahl method

Estimating the leaves properties

Broadband greenness vegetation indices

The broadband greenness VIs were developed for estimating the leaf properties. We used multispectral reflectance values (NIR_a = 800–810 nm; NIR_b = 770–785 nm; red edge = 690–740 nm, and red = 660–675 nm) from the spectrometer, and normalized intensity (*r*, *g*, *b*) of the three bands (*R*, *G*, *B*) from the standard 8-bit digital camera. The normalized intensity is obtained from the digital number (DN) ratio of the corresponding band to sum of the DNs of three bands (Eqs. 4–6).

$$r = \frac{R}{R + G + B} \tag{4}$$

$$g = \frac{G}{R + G + B} \tag{5}$$

$$b = \frac{B}{R + G + B} \tag{6}$$

Comparison of broadband greenness vegetation indices

In the assessment of the 600 images of Robusta coffee plants, 15 indices were derived from a digital camera and 5 indices from the spectrometer (Table 2).

Data analysis

Data processing and analysis of the JPEG images obtained from the digital camera were performed using IMAGEJ Software. Combination of linear and non-linear regression was used to identify the best fit relationship between each VIs and data from Spectrometer, SPAD-502 Chlorophyll meter, digital camera, and laboratory test.

Results and discussion

The selected broadband VIs are obtained from three different tools (spectrometer, SPAD-502 Chlorophyll meter, and a digital camera). We compared the visual observation, reflectance spectra of spectrometer, RGB intensity of the camera, and the leaf Nitrogen status determined by laboratory test. Figure 2 shows four samples of the leaves appearance from the camera. Critical levels of Nitrogen in Robusta coffee leaves (Table 1) were used as reference to estimate the total Nitrogen fertilizer to be applied; the lower greenness level indicates insufficiency of the Nitrogen in the plant.

Evaluation of three bands used in vegetation indices

Coffee plants are called the “evergreen” plants because their leaves are able to survive longer (about 9–10 months) (ICCRI 1999) compared with cereal crops such as rice and wheat. So the dust or dirt will be more attached to the leaves of coffee plants. If cleaned, the outer layers of leaves (epidermis) are feared to be bruised, which could cause the leaf

Table 2 Summary of vegetation indices

Vegetation index	Formula	Allotment	Reference
Simple Ratio Vegetation Index (SRVI)	$SRVI = \frac{NIRb}{Red}$	Spectrometer	Jordan (1969)
Simple Ration Red Edge (SRRE)	$SRRE = \frac{NIRa}{RedEdge}$	Spectrometer	Gitelson et al. (1996)
Normalized Difference Red Edge (NDRE)	$NDRE = \frac{NIRa - Red}{NIRa + Red}$	Spectrometer	Barnes et al. (2000)
Normalized Difference Vegetation Index (NDVI)	$NDVI = \frac{NIRb - Red}{NIRb + Red}$	Spectrometer	Rouse (1974)
Canopy Chlorophyll Content Index (CCCI)	$CCCI = \frac{NDRE}{NDVI}$	Spectrometer	Eitel et al. (2010)
Normalized Difference Vegetation Index R-GB (NDVI _{rgb})	$NDVI_{rgb} = \frac{(g+b)-r}{(g+b)+r}$	Camera	This study
Normalized Difference Vegetation Index-Green (NDVI _{green})	$NDVI_{green} = \frac{g-r}{g+r}$	Camera	Gitelson et al. (2002)
Soil Adjusted Vegetation Index-Green (SAVI _{green})	$SAVI_{green} = \frac{(1+L) * (g-r)}{(g+r)+L}$	Camera	Li et al. (2010)
Normalized Different Index (NDI)	$NDI = \frac{g-r}{g+r+0.01}$	Camera	Mao et al. (2003)
Green Minus Red (GMR)	$GMR = g - r$	Camera	Wang et al. (2013)
Simple Ratio (SR)	$SR = \frac{g}{r}$	Camera	Wang et al. (2013)
Hue (partially)	$Hue = 120 + \frac{60(B-R)}{[\max(R,G,B) - \min(R,G,B)]}$	Camera	Karcher and Richardson (2003)
Dark Green Color Index (DGCI)	$DGCI = \left[\frac{Hue-60}{60} + \left(1 - \frac{Saturation}{100}\right) + \left(1 - \frac{Brightness}{100}\right) \right]$	Camera	Karcher and Richardson (2003)
Visible Atmospherically Resistant Index (VARI)	$VARI = \frac{g-r}{g+r-b}$	Camera	Gitelson et al. (2002)
Blue-Red Adjusted Vegetation Index (BRAVI)	$BRAVI = \frac{N(g-r)}{(g+r+N)}$	Camera	This study
BRAVI-Simple Ratio (BRAVI-SR)	$BRAVI - SR = \frac{N \times g}{r+N}$	Camera	This study
Enhanced Vegetation Index-Green (EVI _{green})	$EVI_{green} = \frac{2.5 * (g-r)}{g+6*r-7.5*b+1}$	Camera	This study; modified from A. Huete et al. (2002)
Enhanced Vegetation Index 2-Green (EVI _{2green})	$EVI_{2green} = \frac{2.5 * (g-r)}{g+2.4*r+1}$	Camera	This study; modified from Jiang et al. (2008)
Optimized Soil Adjusted Vegetation Index-Green (OSAVI _{green})	$OSAVI_{green} = \frac{1.5 * (g-r)}{(g+r)+0.16}$	Camera	This study; modified from Rondeaux et al. (1996)
Simple Ratio Intensity R-GB (SR _{rgb})	$SR_{rgb} = \frac{r}{g+b}$	Camera	This study

L = correction factor (0.5); N = noise intensity, where noise intensity (N) = $\frac{r+b}{255}$

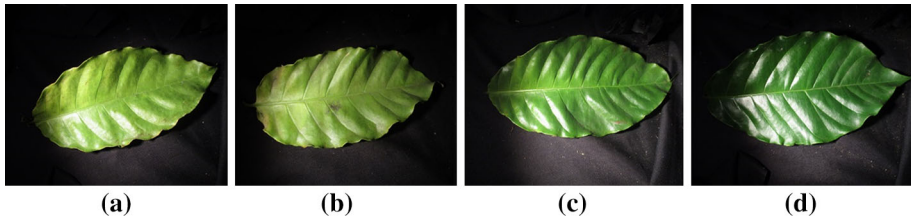


Fig. 2 Robusta leaf visual appearance at different Nitrogen status: **a** deficient, **b** subnormal, **c** normal, **d** high

color change when making observations. In this study, the effect of noise (dirt) and uneven illumination on the leaf surface, indicated by vegetation index value was examined. For the digital camera, the Nitrogen level can be determined by combining R, G and B intensity. Higher Chlorophyll content and Nitrogen level corresponds to a greater G intensity than R intensity. Figure 3 exhibits spectral reflectance of the leaves with different Nitrogen status. Leaves greenness is highly linear with intensity of R band and also linear with Nitrogen levels. Although intensity of the G band can indicate the level of greenness, but this intensity tends to vary within each Nitrogen level. By adding B band intensity could potentially improve the linearity of the G band intensity.

On other hand, B and R band intensity may be used as correction factor for the noise or dirt on the leaf surface. We observed with dirty leaves that B and R band intensity increased and G band intensity decreased. The use of three bands in VIs for direct-leaf measurements using digital camera may reduce the noise using digital camera. It was in line with the study conducted by Gitelson et al. (2002) and Huete et al. (2002) where the use of three bands or adding B band as parameter could be taken as an atmospheric correction while measured from satellites.

Comparison between digital camera and spectrometer

Performance of the digital camera with custom illumination was evaluated by comparing it with spectrometer on individual leaves of the Robusta coffee plants. The results of field

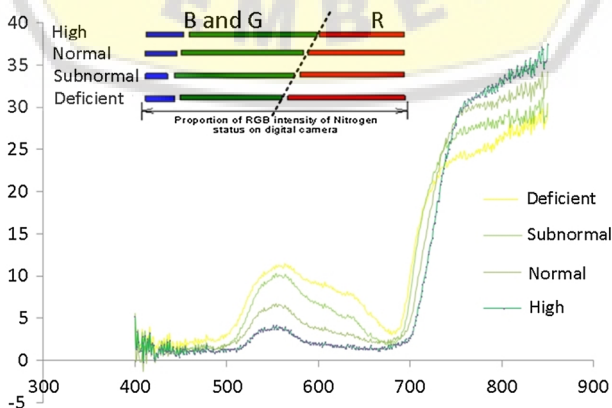


Fig. 3 Spectral reflectance and RGB intensity at different Nitrogen levels

experiments using different tools were examined and compared using best-fit regression at leaf and plant levels (Tables 3, 4).

In this initial analysis, the VIs that comprised three bands performed better than VIs of those comprised two bands. The indices $NDVI_{rgb}$, Hue, BRAVI-SR, and SR_{rgb} highly correlated with NDRE and CCCI of spectrometer in leaf and plant level; where NDRE and CCCI are correlated with photosynthetic pigments and Nitrogen status of the plant (Eitel et al. 2010; Gitelson et al. 1996). Other indices like BRAVI, VARI, and EVI_{green} showed potential in assessing the greenness using direct-leaves measurement.

Estimating photosynthetic pigments

Laboratory test of leaf extraction for estimating the actual value of photosynthetic pigments (Chlorophyll a and b, and Carotenoids) were conducted by Netto et al. (2005), and SPAD-502 Chlorophyll meter were used as independent factor for calibrations. In this study, we evaluated the performance of the digital camera in assessing photosynthetic pigments using direct-leaf measurements. The best-fit models were developed by using best-fit regression between SPAD-502 values and VIs from digital camera.

In calibration (Fig. 4), the VIs comprising three bands performed better than those comprised two bands, with coefficient ranging between $R^2 = 0.798-0.929$ and $R^2 = 0.532-0.899$, respectively. The R^2_{plants} of SR_{rgb} from digital camera was close to NDRE from spectrometer with value of 0.929; and GMR provide the lower value of R^2 in calibration. The best-fit model from each VIs used for further evaluation in estimating Chlorophyll a, Chlorophyll b, and Carotenoids in different levels (leaves and plant).

In evaluation step (Table 5), Hue dominated at leaf levels in estimating Chlorophyll a, Chlorophyll b, and Carotenoid with R^2 -RMSE ($\mu\text{mol m}^{-2}$) of 0.8254–42.086, 0.8205–11.858, and 0.8035–6.634, respectively. While $NDVI_{rgb}$, and BRAVI-SR, SR_{rgb} , dominated at plant level in estimating Chlorophyll a, Chlorophyll b, and Carotenoid with R^2 -RMSE ($\mu\text{mol m}^{-2}$) of 0.93–25.1, 0.93–7.055, and 0.928–3.851, respectively. Overall, the VIs of SR_{rgb} , $NDVI_{rgb}$, Hue, VARI, BRAVI, BRAVI-SR, and EVI_{green} , performed well in estimating photosynthetic pigments; and the particular indices of SR_{rgb} and $NDVI_{rgb}$ slightly better than spectrometer at plant level. For further evaluation, the selected 7 VIs of digital camera and 2 VIs of spectrometer were used in estimating Nitrogen levels of Robusta coffee plants.

Estimating Nitrogen level

Non-destructive method has potential in assessing Nitrogen critical levels plants, and can be used as an indicator for recommending Nitrogenous fertilizer, like the use of digital camera (Ji-Yong et al. 2012; Lee and Lee 2013), spectrometer (Li et al. 2016), and SPAD-502 Chlorophyll meter (Gaju et al. 2016). SPAD uses absorption approach (Balasubramanian et al. 2000; Jinwen et al. 2009; Vesali et al. 2015) while spectrometer and camera use reflectance approach (Samseemoung et al. 2012). In this step, we developed the models in estimating Nitrogen critical level and calibrated using best-fit regression with destructive laboratory results and selected 7 VIs ($NDVI_{rgb}$, Hue, VARI, BRAVI, BRAVI-SR, EVI_{green} , and SR_{rgb}) of digital camera. SPAD-502 Chlorophyll meter and spectrometer (NDRE and CCCI) were used as benchmark in identifying Nitrogen critical levels while destructive laboratory test was used for obtaining the actual value of Nitrogen critical levels.

We examined (Fig. 5) the range of R^2 (R^2 leaves and R^2 plants) of the selected VIs of digital camera. In calibration step, satisfactorily results were obtained with R^2 ranging

Table 3 Coefficient determination (R^2) between digital camera and spectrometer (n = 600) at leaves level

Variables	Digital camera														
	NDVIrgb	SR	NDVI green	GMR	SAVI green	OSAVI green	NDI	Hue	DGCI	VARI	BRAVI	BRAVI-SR	EVI green	EVI green 2	SR rgb
Spectrometer															
SRVI	0.760	0.721	0.826	0.424	0.816	0.823	0.826	0.844	0.635	0.846	0.852	0.816	0.812	0.812	0.8442
SRRE	0.798	0.712	0.770	0.376	0.752	0.763	0.769	0.848	0.730	0.821	0.805	0.798	0.791	0.751	0.8425
NDRE	0.851	0.774	0.788	0.480	0.773	0.782	0.787	0.859	0.746	0.834	0.825	0.848	0.829	0.770	0.8536
NDVI	0.826	0.781	0.852	0.638	0.845	0.850	0.854	0.871	0.615	0.866	0.866	0.843	0.856	0.844	0.8275
CCCI	0.812	0.720	0.725	0.409	0.708	0.718	0.725	0.814	0.736	0.780	0.762	0.802	0.790	0.706	0.812

Table 4 Coefficient determination (R^2) between digital camera and spectrometer (n = 40) at plant level

Variables	Digital camera														
	NDVI rgb	SR	NDVI green	GMR	SAVI green	OSAVI green	NDI	Hue	DGCI	VARI	BRAVI	BRAVI- SR	EVI green	EVI green 2	SR rgb
SRVI	0.845	0.849	0.942	0.747	0.941	0.942	0.942	0.909	0.747	0.936	0.905	0.844	0.834	0.941	0.9292
SRRE	0.889	0.869	0.899	0.613	0.891	0.895	0.899	0.913	0.830	0.921	0.924	0.894	0.888	0.895	0.927
NDRE	0.939	0.926	0.917	0.729	0.910	0.914	0.917	0.925	0.854	0.935	0.924	0.937	0.925	0.913	0.9393
NDVI	0.953	0.924	0.929	0.843	0.933	0.931	0.929	0.967	0.744	0.967	0.97	0.856	0.947	0.927	0.955
CCCI	0.937	0.910	0.896	0.655	0.886	0.892	0.895	0.921	0.871	0.923	0.905	0.932	0.924	0.890	0.9342

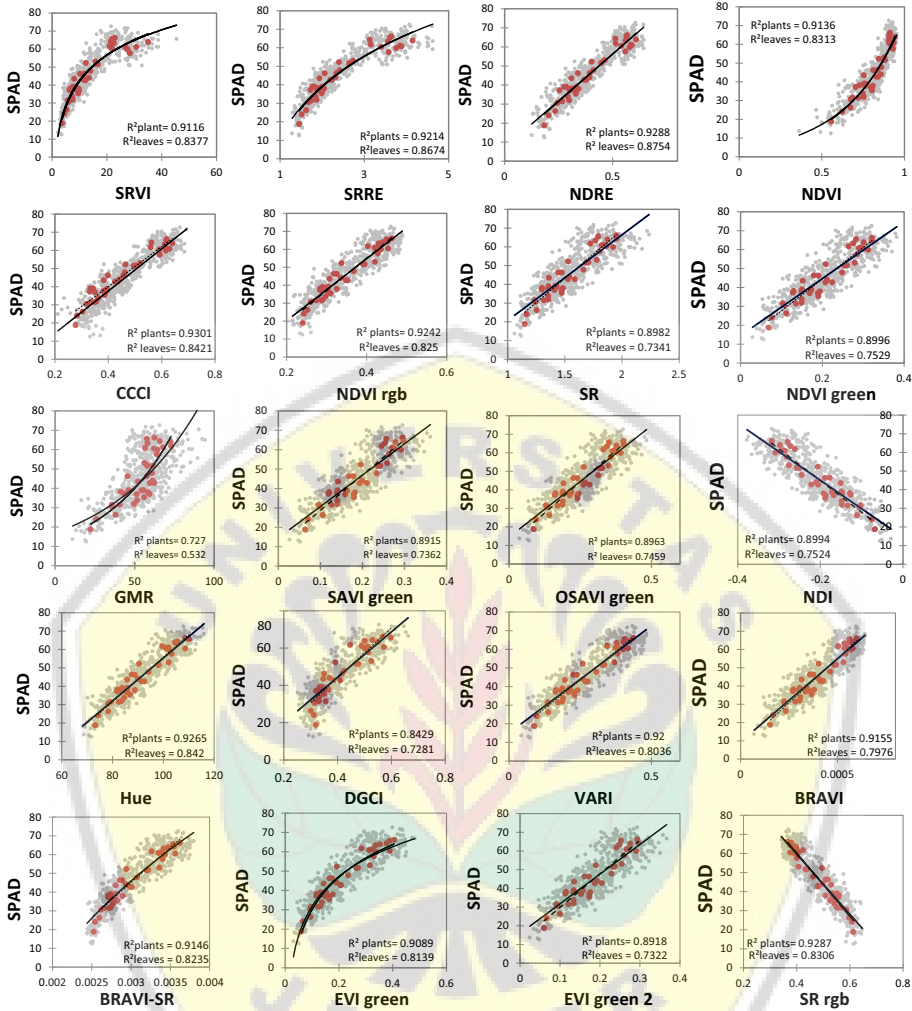


Fig. 4 Best-fit regression using 600 leaves and 40 plants samples from VIs (*dashed line and straight line* are corresponding to R^2_{plant} and R^2_{leaves} respectively)

between 0.689 and 0.811, while SPAD-502 Chlorophyll meter and spectrometer provided R^2 ranging between 0.701–0.821 and 0.719–0.854, respectively. These results indicated strong linear relation with SPAD-502 and spectrometer. The selected model of each VIs was further evaluated at leaf and plant levels.

Evaluation of Nitrogen level

Estimation performance of the models was further evaluated by proportion of the samples in each critical Nitrogen level between each non-destructive method and the laboratory results. Beside R^2 and RMSE, other two factors are considered in this evaluation process of the models toward to the actual Nitrogen value; the first factor is the distribution or proportion ratio (D(%)) of critical Nitrogen level obtained from non-destructive

Table 5 Determination coefficients (R^2), and root mean square errors (RMSE; $\mu\text{mol m}^{-2}$) of best-fitted regression of Chlorophyll a and b, and Carotenoids obtained from several indices of spectrometer and digital camera using cross-validation of SPAD-502 at leaves ($n = 600$) and plant ($n = 40$) levels

Vegetation index	Equation	n = 600 Leaves						n = 40 Plants																
		Chlorophyll a			Chlorophyll b			Carotenoids			Chlorophyll a			Chlorophyll b			Carotenoids							
		R^2	RMSE		R^2	RMSE		R^2	RMSE		R^2	RMSE		R^2	RMSE		R^2	RMSE		R^2	RMSE			
Spectrometer																								
SRVI	$\text{SPAD} = 20.185\ln(x) - 3.7733$	0.8112	43.762	0.8011	12.483		0.776	7.083	0.849	31.021	0.885	9.046	0.867	5.178										
SRRE	$\text{SPAD} = 39.833\ln(x) + 11.802$	0.8521	38.724	0.8506	10.82		0.8377	6.079	0.917	27.348	0.914	7.801	0.9071	4.36										
NDRE	$\text{SPAD} = 97.959x + 7.2816$	0.8629	37.287	0.8625	10.381		0.851	5.776	0.927	25.795	0.924	7.34	0.918	4.092										
NDVI	$\text{SPAD} = 4.0305e^{2.9166x}$	0.8153	43.279	0.8093	12.225		0.7904	6.852	0.898	30.384	0.893	8.700	0.885	4.858										
CCCI	$\text{SPAD} = 116.23x - 8.8546$	0.8421	40.021	0.8476	10.928		0.8432	5.926	0.933	24.635	0.932	6.935	0.928	3.827										
Digital camera																								
NDVI rgb	$\text{SPAD} = 172.62x - 14.274$	0.8089	44.023	0.8029	12.427		0.7835	6.964	0.929	25.459	0.929	7.105	0.927	3.852										
NDVI green	$\text{SPAD} = 150.8x + 14.504$	0.72	53.287	0.706	15.177		0.675	8.527	0.897	30.605	0.894	8.667	0.889	4.773										
SAVI green	$\text{SPAD} = 161.6x + 14.56$	0.701	55.092	0.6854	15.701		0.6528	8.818	0.887	32.064	0.883	9.104	0.876	5.029										
OSAVI green	$\text{SPAD} = 120.11x + 14.519$	0.7119	54.052	0.6973	15.399		0.666	8.650	0.893	31.213	0.890	8.850	0.884	4.880										
NDI	$\text{SPAD} = -152.64x + 14.505$	0.7194	53.343	0.7054	15.193		0.6747	8.536	0.896	30.649	0.894	8.681	0.888	4.781										
GMR	$\text{SPAD} = 16.995e^{0.0174x}$	0.36	80.598	0.327	22.959		0.278	12.714	0.618	58.889	0.597	16.917	0.564	9.441										
SR	$\text{SPAD} = 45.694x - 24.987$	0.6945	55.667	0.678	15.885		0.644	8.926	0.897	30.575	0.894	8.655	0.888	4.780										
Hue	$\text{SPAD} = 1.1628x - 60.916$	0.8254	42.086	0.8205	11.858		0.8035	6.634	0.924	26.253	0.922	7.448	0.917	4.116										
DGCI	$\text{SPAD} = 121.55x - 4.2019$	0.727	52.622	0.7258	14.658		0.713	8.022	0.856	36.114	0.858	10.036	0.856	5.422										
VARI	$\text{SPAD} = 115.05x + 15.054$	0.78	47.273	0.77	13.406		0.747	7.525	0.922	26.538	0.922	7.437	0.920	4.045										
BRAVI	$\text{SPAD} = 90638x + 9.5061$	0.777	47.596	0.769	13.467		0.746	7.540	0.916	27.663	0.915	7.779	0.912	4.237										
BRAVI-SR	$\text{SPAD} = 109.41\ln(x) + 681.54$	0.803	44.667	0.796	12.63675		0.776	7.090	0.927	25.684	0.928	7.170	0.926	3.887										
EVI green	$\text{SPAD} = 22.187\ln(x) + 82.984$	0.8057	44.394	0.8033	12.413		0.788	6.887	0.919	27.044	0.919	7.593	0.917	4.117										
EVI green 2	$\text{SPAD} = 158.52x + 15.935$	0.695	55.613	0.679	15.859		0.657	8.910	0.887	32.011	0.883	9.094	0.876	5.030										
SR rgb	$\text{SPAD} = -157.91x + 122.84$	0.816	43.215	0.810	12.193		0.791	6.837	0.930	25.127	0.930	7.055	0.928	3.851										

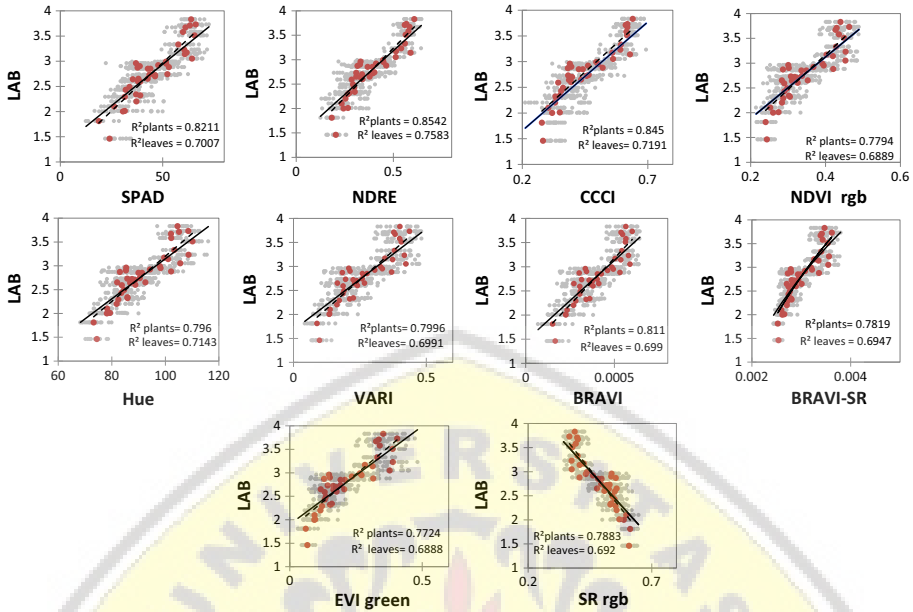


Fig. 5 The best-fit regression models between laboratory tests and different tools and indices (*dashed line* and *straight line* are corresponding to R^2_{plant} and R^2_{leaves} respectively)

observation and the laboratory results in each Nitrogen level category (NLC). The second factor is validity ratio ($V(\%)$) obtained from total samples accurately estimated divided by total samples in all Nitrogen level category. These ratios are as shown in equations (Eqs. 7–9).

$$D = 100\% \times \sum_{\text{Level}=\text{Deficient}}^{\text{High}} \left(1 - \frac{|\sum \text{Sample}_{\text{Actua}}(\text{Level}) - \sum \text{Sample}_{\text{Prediction}}(\text{Level})|}{\text{Total Sample}} \right) \quad (7)$$

$$V = 100\% \times \frac{\sum \text{NLC Sample}_{(\text{Prediction})}}{\text{Total Sample}} \quad (8)$$

where $\text{NLC Sample}_{(\text{Prediction})}$ obtained from the following algorithm:

If $\text{NLC Sample}_{(\text{Prediction})} = \text{NLC Sample}_{(\text{Actual})}$

Then

$$\text{NLC Sample}_{(\text{Prediction})} = 1 \quad (9)$$

Else

$$\text{NLC Sample}_{(\text{Prediction})} = 0$$

Performance evaluation of the 10 selected models obtained from SPAD-502 Chlorophyll meter, spectrometer, and digital camera to estimate Nitrogen status at individual leaf, and at plant levels are presented in Tables 6 and 7 respectively.

In Table 6, Hue provided higher R^2 and RMSE within SPAD-502 Chlorophyll meter and other indices from camera, but the performances of validity and proportion ratios were

Table 6 Performance evaluation of the models in estimation of Nitrogen status at leaves level (n = 600 leaves)

L/AB	Model	Leaves with different Nitrogen level				Performance			R ²	RMSE (% Nitrogen)
		Deficient 15	Subnormal 240	Normal 240	High 105	V (%)	D (%)			
							High	D		
SPAD	LAB = 0.0329x + 1.2932	7	277	206	110	68.3	86	0.678	0.305	
Spectrometer	NDRE	LAB = 3.5858x + 1.392	0	303	172	125	73.17	72.3	0.757	0.265
	CCCI	LAB = 4.2243x + 0.8155	2	294	179	125	72.3	75.3	0.718	0.286
	Camera									
NDVI _{rgb}	LAB = 6.204x + 0.6428	0	305	184	111	65.17	76.3	0.689	0.300	
Hue	LAB = 0.0474x - 1.5418	5	292	170	133	64.3	67.72	0.714	0.288	
VARI	LAB = 4.8295x + 1.5113	2	298	175	125	64	74	0.698	0.295	
BRABI	LAB = 3861x + 1.2478	16	270	192	122	61.83	84	0.698	0.296	
BRABI-SR	LAB = 3.9522ln(x) + 25.767	0	314	176	110	64.83	73.67	0.695	0.297	
EVI _{green}	LAB = 4.6995x + 1.7966	0	319	155	126	64.3	66.67	0.689	0.300	
SR _{rgb}	LAB = -5.6687x + 5.5675	0	293	196	111	64.17	80.3	0.692	0.298	

Value in bold face refers to the highest R² in the respective category (Camera)

Table 7 Performance evaluation of the models in estimation of N status at plants level (n = 40 plants)

L:AB	Model	Plants with different of Nitrogen level						Performance			R ²	RMSE (% Nitrogen)
		Deficient		Subnormal		Normal		High	V (%)	D (%)		
		1	16	16	16	7						
SPAD	LAB = 0.0329x + 1.2932	0	20	13	7	75	80	0.821	0.233			
Spectrometer	NDRE	LAB = 3.5858x + 1.392	0	20	11	9	80	0.854	0.21			
	CCCI	LAB = 4.2243x + 0.8155	0	23	8	9	77.5	0.845	0.217			
	Camera											
NDVI _{rgb}	LAB = 6.204x + 0.6428	0	21	11	8	75	70	0.779	0.259			
Hue	LAB = 0.0474x - 1.5418	0	21	11	8	75	70	0.796	0.249			
VARI	LAB = 4.8295x + 1.5113	0	21	9	10	75	60	0.8	0.247			
BRABI	LAB = 3861x + 1.2478	0	20	10	10	72.5	65	0.810	0.24			
BRABI-SR	LAB = 3.9522ln(x) + 25.767	0	21	11	8	75	70	0.782	0.257			
EVI _{green}	LAB = 4.6995x + 1.7966	0	23	8	9	72.5	55	0.772	0.263			
SR _{rgb}	LAB = -5.6687x + 5.5675	0	21	12	7	72.5	75	0.788	0.253			

Value in bold face refers to the highest R² in the respective category (Camera)

lower than $NDVI_{rgb}$ and BRAVI, respectively. In addition, Table 7 shows that BRAVI provided higher R^2 and RMSE than other VIs obtained from digital camera and validity was ratio close to other VIs, while the proportion ratio of SR_{rgb} was better than BRAVI. These ratios are useful to identify the spread of the samples used and identifying the validity of different category of Nitrogen level.

Another example in Table 7, although CCI provided good R^2 and RMSE, the proportion ratio was the lowest in respective category due to a large gap in sum of samples between prediction and actual Nitrogen level. The validity ratio closely followed the R^2 , which is more precise in predicting the category of Nitrogen level. Overall, for spectrometer (Tables 6, 7), NDRE provided the highest value of R^2 and lowest value of RMSE. This is in line with the study of Song et al. (2016) where NDRE was significantly correlated with Nitrogen level of the plant. The use of spectrometer provides more precise estimation than SPAD-502 and digital camera at leaf and plant levels as indicated by better value of R^2 and RMSE in estimating Nitrogen critical level.

Although the selected VIs obtained from digital cameras were highly correlated with actual Nitrogen levels, the Hue index provided uniqueness in measurement. The use of partial Hue equation (Table 2) could cover the greenness related to photosynthesis pigments and Nitrogen estimation. Overall performance of $NDVI_{rgb}$, SR_{rgb} , Hue, VARI, BRAVI, BRAVI-SR, and EVI_{green} are reasonably close to that of SPAD-502 Chlorophyll meter, spectrometer and actual values using laboratory measurement. This exhibits a proven potential in developing a low-cost ground based remote sensing method using direct-leaf measurements for Robusta coffee plants. With their higher estimation performance, these VIs could be potentially implemented in estimating of photosynthesis pigments and Nitrogen.

Conclusion

This study evaluated the potential of using a digital camera as a low-cost system and compared it with the spectrometer and SPAD-502 Chlorophyll meter in estimating Chlorophyll a and b, Carotenoids, and Nitrogen critical levels of Robusta coffee plants using direct-leaf measurements under different field conditions. Several VIs are developed, modified, and compared with the existing VIs. The following can be drawn as key conclusions:

1. We claimed that the use of three bands in VIs offered significantly better results than those comprised two bands.
2. $NDVI_{rgb}$ and SR_{rgb} are proposed as alternative indices in place of $NDVI_{green}$ and SR, respectively, at leaf level.
3. $NDVI_{rgb}$, SR_{rgb} , Hue, VARI, BRAVI, BRAVI-SR, and EVI_{green} are recommended for estimating biophysical properties like Chlorophyll a and b, Carotenoids, and Nitrogen level of the broadleaf plant like Robusta coffee through direct-leaf measurements.
4. These VIs values are closely related to the spectrometer and SPAD-502 Chlorophyll meter values at leaf and plant levels.

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