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Comparing Visible Light Based Vegetation Index and Chlorophyll Meter to Estimate Chlorophyll and Nitrogen Content of Tea (*Camellia Sinensis* L. Kuntze) Leaves

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ABSTRACT

Leaf chlorophyll content is widely used as an indicator of tea leaves quality as well as plant nitrogen status. The development of a rapid and low-cost estimation method will considerably useful to the management of sustainable crop production. This research was aimed to develop a new method usinguncalibrated visible light images captured by a commercial camera mounted on an Unmanned Aerial Vehicle (UAV) and compared them to chlorophyll meter SPAD-502. The study used B*A* simple difference vegetation index (BAVI) which derived from L*a*b data transformation of uncalibrated RGB images captured from a UAV. Data was collected from an experiment of nitrogen dosage levels on 3 years after prunning tea crops. The result showed that the regression of BAVI values with shoots' chlorophyll content were shown by linear equations $y = 0.0025x + 0.9816$with determinant coefficients (R^2) = 0.8012 ($P<0.01$), while its regression with nitrogen content of shoots was $y = 0.0045x + 2.982$ with $R^2 = 0.6341$($P<0.01$). The maximum bias of the measurement with 95% confidence was 4.14% (foliar chlorophyll content measurement) and 2.73% (foliar nitrogen content measurement) of the SPAD-502 method.

Keywords
nitrogen fertilizing, unmanned aerial vehicle, visible light spectrum

INTRODUCTION

Leaf chlorophyll content is an important indicator of quality both in the production of green and black tea (*Camellia sinensis* L. Kuntze) (Ošťádalová*et al.*, 2014; Gogoi and Borua, 2017). The quantitative measure of the actual content of tea leaves chlorophyll is not only useful to assess the quality of tea harvest, but also it provides the possibility to apply nitrogen fertilizer more precisely. Those possibility comes from the relationship between chlorophyll and nitrogen. It was reported that foliar chlorophyll and nitrogen content estimation using a chlorophyll meter has improved real-time paddy N management and grain yield estimation so it can be reliably exploited in precision agriculture of paddy fields (Gholizadeh*et al.*, 2017).
There are various techniques to measure foliar chlorophyll content have been developed by many researchers such as laboratory based measurement, chlorophyll meter (Dong *et al.*, 2019), and image based estimation (Li and He, 2008; Prado Osco *et al.*, 2019; de Souza *et al.*, 2020; Osco *et al.*, 2020). Destructive laboratory-based measurement methods of crop chlorophyll contents are time-consuming and complicated. Chlorophyll meter provides a rapid and easy method to non-destructive assess foliar chlorophyll contents in various crops, such as sorghum (Yamamoto *et al.*, 2002), rice (Pushpanathan, Kumar and Siddeswaran, 2004; Gholizadeh*et al.*, 2009; Jinwen*et al.*, 2015; Yuan *et al.*, 2016), tea (Liu, Yang and Yang, 2012), corn (Dong *et al.*, 2019). While it

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provides a rapid method, the use of a chlorophyll meter can only estimate samples' chlorophyll contents. The development of unmanned aerial vehicle (UAV) opens possibilities to develop a fast leaves chlorophyll content estimation based on aerial digital images non-destructively (Candiago*et al.*, 2015; NaveedTahir*et al.*, 2018; Roosjen*et al.*, 2018; SimicMilas*et al.*, 2018; Prado Osco *et al.*, 2019; Cao *et al.*, 2020; Guo *et al.*, 2020; Kim *et al.*, 2020). It means that the use of such a method can quickly provide chlorophyll and nitrogen estimates of all plants covered in the images. It was reported that Normalized Difference Vegetation Index (NDVI) derived from a multispectral sensor images captured from a UAV can adequately assess the N status of turfgrasses and its spatial variability of a very large area (Caturegli*et al.*, 2016).

In term of practical usages, the use of multi- or hyper-spectral sensors are quite expensive and requires high expertise in image acquisition and analysis (Aasen*et al.*, 2015). A multi- or hyper-spectral sensors comprises more than three channels and possibly includes non-visible light spectrum. A visible light based digital camera, on the other hand, records images in a three channels RGB color space format which contains information of red, green and blue reflectance. The development of the UAV industry makes it possible now for laypersons to take a very high spatial resolution georeferenced RGB images easily at low cost. Recently several studies have been conducted to utilize digital camera with a visible light sensor as a tool to quantify the greenness of leaves and correlate them with leaf chlorophyll content and plant nitrogen status (Wang *et al.*, 2014; Bendig, 2015; Bareth*et al.*, 2016; Li *et al.*, 2018). It was reported that vegetation index (VI) derived from uncalibrated RGB images produces a promising range of values representing the observable variability of crop growth when the NDVI derived from calibrated images was saturated (Bareth*et al.*, 2016).

The proposed method of this research implemented a different approach on using uncalibrated RGB images to estimates foliar chlorophyll and nitrogen content of tea. Instead of using the RGB value to derive VI, we transform the RGB value into the CIE L*a*b* color space before calculating VI. CIE L*a*b* is a device independent color space format which decouples the lightness value of RGB colors into L value while a* value and b* value record the color. The proposed VI were calculated based on a* and b* values so that the derived VIs were expected to be better in term of sensitivity. The success of the proposed method will dramatically help improve the speed and reduce the cost of tea leaf chlorophyll and nitrogen content estimation. The method also has the potential to be applied to improve the efficiency of N fertilizers usage which will be necessary for sustainable tea crop production. The aim of this study was to evaluate the use of VI derived from uncalibrated digital aerial images captured from a UAV to estimate chlorophyll and nitrogen content of tea leaves and comparing them with SPAD-502 chlorophyll meter.

MATERIALS AND METHODS

Site and Experiment Design

The research was carried out at the Wonosari Tea Plantation PTPN XII, Lawang, Malang Regency, East Java, Indonesia with a geographical position -07° 49' 17.6" (latitude) 112° 38' 36" (longitude). The altitude of the plantation is 905-1050 meters above sea level. The soil type of field is dominated by Andosol. The temperature ranges from 15^{o} to 28^{o}C, and the rainfall ± 3000 mm. The experiment was conducted from February to May 2015.

Data were collected from an N-dose experiment comprised of 6 levels of treatment with 3 replications. Each of plots were 18 x 27 meters^2 which selected from homogeneous 3 years old after pruning productive crop and arranged in a Completely Randomized Design. The level of

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treatment of nitrogen doses was determined based on the percentage to the dose applied by the companyat the experiment field, namely: 0 (control), 25, 50, 75, 100 and 125%. The reference dose used by the company was 350 Kg N.Ha^{-1}.year^{-1}(Effendi *et al.*, 2011) which was applied 4 times a year or a quarter of the recommended dosage at each application. The levels of the treatments, thus, consisted of 0 Kg N/Ha, 21.9 Kg N/Ha, 43.8 Kg N/Ha, 65.6 Kg N/Ha, 87.5 Kg N/Ha, and 109.4 Kg N/Ha. Furthermore, the dose was divided by the plant population per hectare to determine the dosage of fertilizer that will be applied to each plant in the experimental plot. Fertilizer application was carried out 32days before the first observation, after picking time by burying it in 4 separate points, each of which was 60 cm apart from the stem.

SamplingandObservations

Observations were made on 32 and 45days after treatment (DAT) starting on March 26, 2015 for the first observation. On each trial plot, samples of plant shoots were marked with a marker frame that could be seen from a camera mounted on an unmanned aircraft. The sample frame was shaped with a + sign with a hole in the middle. The size of the hole is 25 x 25 cm. The shoot leaf samples were selected inside the marker frame. Aerial photo recording was carried out using a Canon S100 camera mounted on an unmanned aircraft (3DRobotics Iris). The aircraft was programmed to fly across the path above the experimental field at an altitude of 75 meters above ground level. The flight path programming was done using Mission Planner software (Oborne, 2012). Spectral data was taken from marked pixels from each sample. Aerial photo recording was carried out between 8:00 and 10:00 am.

The measurements by chlorophyll meter were immediately carried out after the image recording. Leaf samples were shoots taken from each of the sample markers. Measurements were carried out on each sample leaf at 3 points: (i) at the base of the leaf (1/3 of the length of the leaf); (ii) the center of the leaf (1/3 the middle); and at the end of the leaf (1/3 part of the end). The reading was done on each face of the leaf so that 6 observations are obtained which were then averaged to get the SPAD reading value from 1 single leaf.

The sample of leaves was brought right away after the SPAD observation to the laboratory in an ice cube filled styrofoam box. The foliar chlorophyll content of tea measured destructively in the laboratory (Chl_{Lab}) was carried out using Arnon method. The sample leaves were then analyzed directly or frozen at -20^{o}C until it was analyzed. The leaves were dried at 105^{o} Celsius for 30 minutes to quickly stop polyphenol oxidase enzyme activities and then at 70^{o}C until a stable dry weight was reached. Dried leaf was, then, crushed and the N content was calculated using the Kjehldahl method (Wang *et al.*, 2017). Measurements were carried out 3 times each and then averaged as a value of 1 sample. Destructive chlorophyll content measurement was carried out using Arnon method. Samples of five fresh leaves from each observation point were homogenized with 80% ethanol. After centrifugation, the residue was re- extracted with 80% acetone. The supernatants then were combined and their chlorophyll concentrations determined by the Arnon method(Rajalakshmi and Banu, 2015).

Determination of nitrogen concentration destructively in the laboratory (N_{Lab}) was carried out first by drying the leaves of the sample at 105^{o}C for 30 minutes so that the enzyme activity of polyphenol oxidase stopped quickly. Subsequent drying was carried out at 70^{o}C until a stable dry weight was achieved. The dried leaves were crushed and the N content is calculated using the Kjehldahl method (Wang *et al.*, 2017). Measurements were made 3 times from every sample which then be averaged as a value of 1 sample observation.

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Data processing and statistical analysis

The reading of digital values on the red, green, blue (RGB) channel of aerial photography was done using the Fiji Image Processing software (Schindelin*et al.*, 2012). The digital data is extracted from the leaves of previously marked samples that were the same as those used for destructive analysis in the laboratory. The digital RGB value data was then converted to the XYZ color space and finally, L*a*b* using the method as applied by Zhang*et al*.(Zhang *et al.*, 2006). A simple difference between the b* and a* values was used to determine the B*-A* vegetation index (BAVI) which calculated following the formula BAVI = b*-a*.

Foliar chlorophyll and nitrogen content estimation model for each investigated method was developed by implementing regression analysis between the predictors and destructive laboratory measurements of nitrogen and chlorophyll using data from 32 DAT observations. Foliar chlorophyll and nitrogen content estimation using BAVI (Chl_{BAVI} and N_{BAVI}) were obtained by applying BAVI regression equation to 45 DAT observation data. Foliar chlorophyll and nitrogen content estimation using SPAD-502 (Chl_{SPAD} and N_{SPAD}) were obtained by applying regression equation to 45 DAT observation data. Comparisons between measurement methods were performed by investigating the agreement of the values of each methods using the Bland-Altman comparison analysis(Bland and Altman, 1986).All statisticalanalyses were carried out using SPSS software.

RESULT AND DISCUSSION

Nitrogen and chlorophyll content estimation models

Foliar nitrogen and chlorophyll content estimation models were developed by implementing regression analysis on BAVI and SPAD readings against destructive laboratory measurement of leaf nitrogen and chlorophyll content using data from 32 DAT observations. Figure 1 presents the results of the regression analyses. The regression lines suitable for modeling the relationship of BAVI values with shoots' chlorophyll content were shown by linear equations $y = 0.0025x + 0.9816$with determinant coefficients (R^2) = 0.8012 significantly at $P<0.01$ (Figure 1A). The relationship of BAVI values with nitrogen content of shoots follows a linear pattern with the equation $y = 0.0045x + 2.982$ with $R^2 = 0.6341$ significantly at $P<0.01$ (Figure 1B). Both of these equations indicate that anincrease ofBAVI value will be followed by an increase in chlorophyll content or nitrogen content of shoots of tea plants.

The regression line describing the relationship between SPAD readings and tea leaf chlorophyll content was presented by linear equation $y = 0.0371x - 0.1557$ with $R^2 = 0.8784$ significantly at $P<0.01$ (Figure 1C). The regression analisys of SPAD readings and tea shoot nitrogen content also resulted a significant ($P<0.01$) linear equation of $y = 0.0442x + 1.7075$ with $R^2 = 0.5876$. While all regression analyses resulted significant models, it was unfair to use their coeeficient correlation to compare whether one method can be applied interchangeably with the others (Bland and Altman, 1986). The comparison of the two methods will be discussed in the next section.

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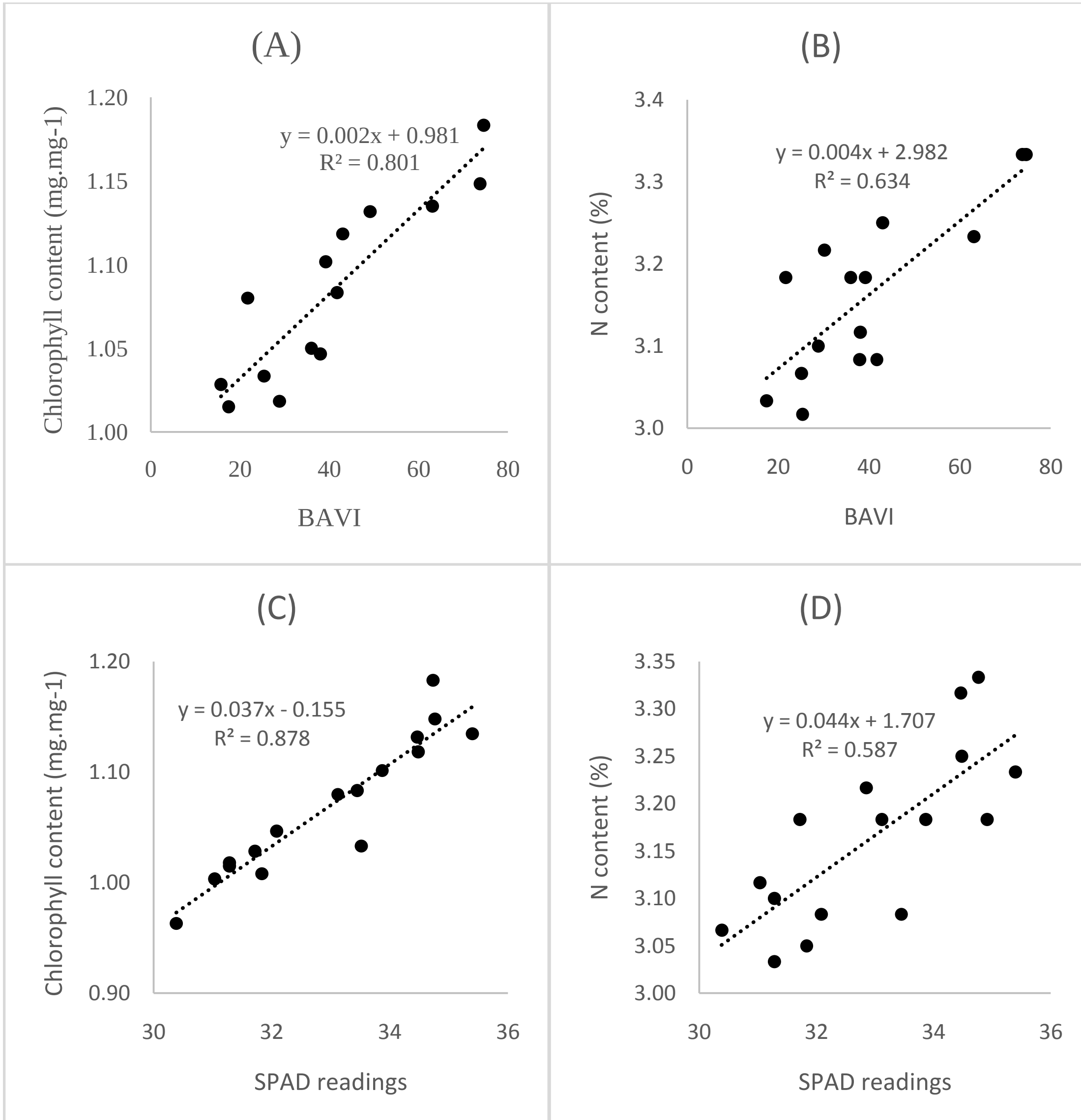

Figure 1.Relationship between the BAVI and content of chlorophyll (A); the BAVI and nitrogen conten (B); SPAD readings and chlorophyll content (C) andSPAD readings and content of N of tea shoots.

Comparison of Estimation Methods

Comparison of the measurement methods was carried out on foliar tea chlorophyll and nitrogen estimation data which came from the application of previously developed models on 45 DAT observation data. Table 1 presents descriptive statistic of the predictors and foliar chlorophyll and N content measured by each method. All variables' mean and median look similar and could be interpreted that the data of all variables are symmetric. The means (± SD) varied by methods and subjects of measurement, from 1.08 mg.mg^{-1} (± 0.045 mg.mg^{-1}) to 1.10 mg.mg^{-1} (± 0.046 mg.mg^{-1}) for chlorophyll and 3.16% (± 0.068%) to 3.18% (± 0.055%) for foliar nitrogen content. The BAVI based measurement produced a narrower range of measurement on foliar chlorophyll content (0.12 mg.mg^{-1}) but a slightly wider on N content (0.19%) than estimation using SPAD (0.17 mg.mg^{-1} for chlorophyll and 0.16% for N content.

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Tabel 1.Descriptive statistic of the estimators and measured data from 45 DAT observations.

	BAVI	SPAD	Chl_{BAVI} (mg.mg^{-1})	Chl_{SPAD} (mg.mg^{-1})	N_{BAVI} (%)	N_{SPAD} (%)
Mean	44.01	33.35	1.09	1.08	3.18	3.17
Median	42.28	33.43	1.09	1.08	3.16	3.17
Variance	171.33	1.47	0.001	0.002	0.003	0.002
SD	13.09	1.21	0.033	0.045	0.055	0.049
Minimum	21.42	31.45	1.04	1.01	3.08	3.09
Maximum	70.72	36.00	1.16	1.18	3.27	3.26
Range	49.30	4.55	0.12	0.17	0.19	0.16

Chl_{BAVI} = Chlorophyll estimated using BAVI; Chl_{SPAD} estimated using SPAD-502; N_{BAVI} = N estimated by BAVI; N_{SPAD} = N estimated by SPAD.

Figure 2A and 2C presents the scatter diagrams of Chl_{SPAD} vs. Chl_{BAVI} and N_{SPAD}-N_{BAVI} respectively.The line of equality (represented by the diagonal line on the scatter plots) described the perfect agreement i.e. the estimated values of both methods are exactly the same. Both figures show that all of the estimated values from different method were correlated each other. The results indicated that foliar chlorophyll content measured with BAVI associated with the one measured by SPAD-502, while two tail paired t-Test for Chl_{SPAD} vs. Chl_{BAVI} indicated that the values of each measurement method were not significantly different (P(T<=t) = 0.15). Tea shoot N content estimated with BAVI were associated with the one measured by SPAD-502 where two tail paired t-Test indicated that N_{SPAD} and N_{BAVI} were not significantly different (P(T<=t) = 0.63). The correlation among the methods and its t-Test result, however, could not indicate the agreement between different measurement methods. Indeed, the scatter plots diagram showed disagreement. If the values came from different methods agreed perfectly, all the paired data point would fall on the equality line. Further analysis was needed to determine both the magnitude and direction of the bias.

Figure 2B and Figure 2D present the Bland-Altman plots for Chl_{BAVI} vs. Chl_{SPAD} comparison and N_{SPAD} vs. N_{BAVI} respectively. The y-axis on the chart represents the difference between the values measured with the two compared methods while the x-axis represents the mean of the values measured by both of the methods. The difference between Chl_{BAVI} and Chl_{SPAD} varied from -0.082 - 0.030 mg.mg^{-1} which was distributed normally (Kolmogorov-Spirnov test showed p = 0.71) while the difference between N_{SPAD} and N_{BAVI} varied from -0.09 to 0.06% which was distributed normally (Kolmogorov-Spirnov test showed p = 0.86). The difference which randomly laid on negative and positive values indicated that there was no systematic bias on both estimation, foliar chlorophyll nor nitrogen content. Neither of the differences was no more likely to be higher or lower at BAVI than at SPAD-502 measurements, so it was not indicating a calculation of percentage error.

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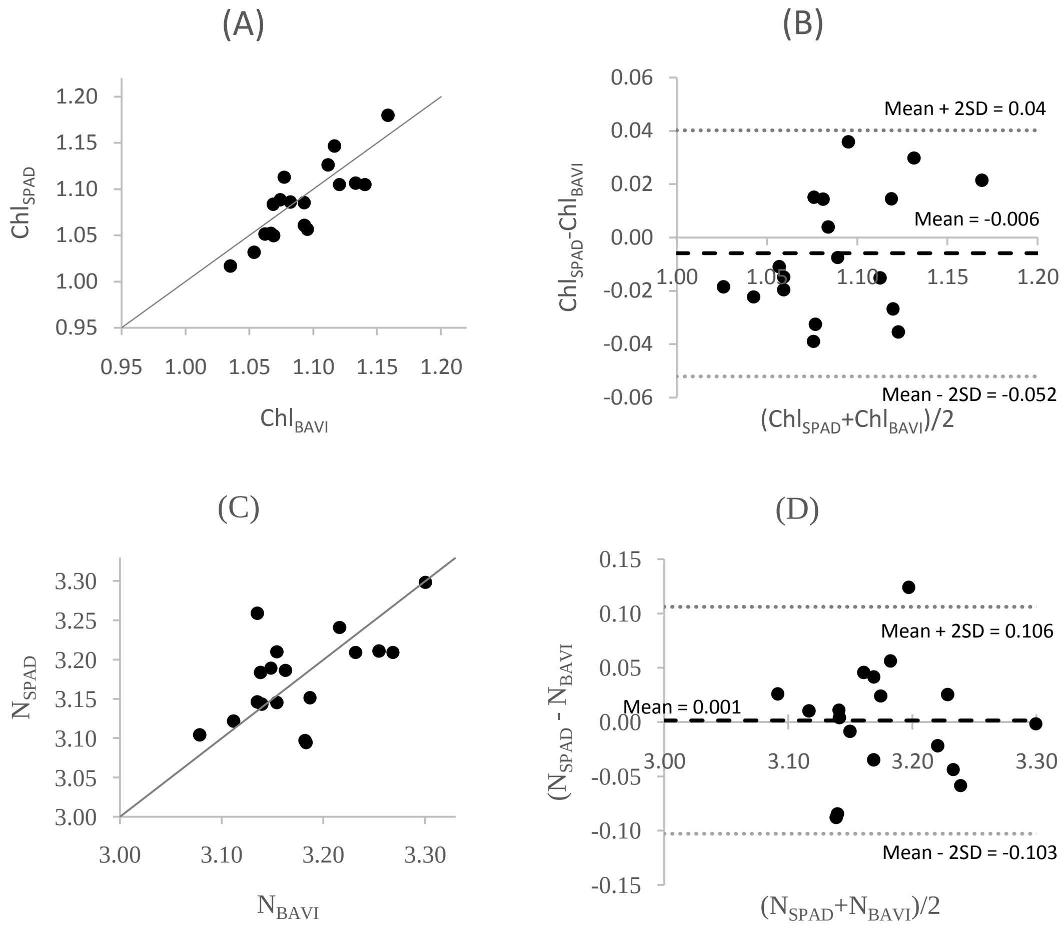

Figure 2. Scatter plot of foliar chlorophyll (A) and nitrogen (C) content estimates using BAVI vs. SPAD and Bland-Altman plots of chlorophyll (B) and nitrogen (D) estimates differences between BAVI and SPAD. Mean + 2SD and mean -2SD lines define 95% limits of agreement

Tabel 2. Bland-Altman plot statistic.

Parameter	Unit	Standart error (*se*)	*t* value (*df*=17)	Confidence	Confidence intervals from	to
Chl_{SPAD} vs. Chl_{BAVI}						
differece mean ($\bar{d}$)	-0.006	0.005	2.11	0.011	-0.017	0.006
standard deviation (*s*)	0.023					
$\bar{d}$-1.96*s*	-0.051	0.009	2.11	0.020	-0.071	-0.031
$\bar{d}$+1.96*s*	0.039	0.009	2.11	0.020	0.019	0.059
N_{SPAD} vs N_{BAVI}						
differece mean ($\bar{d}$)	-0.005	0.010	2.11	0.022	-0.027	0.017
standard deviation (*s*)	0.044					
$\bar{d}$-1.96*s*	-0.092	0.018	2.11	0.038	-0.130	-0.054
$\bar{d}$+1.96*s*	0.081	0.018	2.11	0.038	0.043	0.120

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While the perfect agreement between two compared methods would be indicated by zero difference at both estimates, the mean of measurement difference between compared methods indicates the bias of the measurement (Giavarina, 2015). Table 2 shows the Bland-Altman plot statistic. The mean of the differences,$\bar{d}$, of SPAD and BAVI chlorophyll estimations (Chl_{SPAD} - Chl_{BAVI}), as shown at Table 2, was -0.006 $mg.mg^{-1}$, which means that the estimation made by BAVI tend to be higher than the one made my SPAD by 0.006 $mg.mg^{-1}$. Meanwhile, the$\bar{d}$ of foliar nitrogen estimation made by both methods was -0.005% which mean that N_{BAVI} tent to be higher than N_{SPAD} by 0.005%. Those results showed that BAVI estimation consistently higher than SPAD estimation.
The upper limit (defined as $\bar{d}$+1.96*s*) and the lower limit (defined as $\bar{d}$-1.96*s*) determines the limits of agreement at which 95% of the mean differences between two methods estimates are expected to be laid off as long as they are normally distributed (Bland and Altman, 1986).As presented in Table 2, the limits of agreement between foliar chlorophyll content estimated by SPAD-502 and BAVI was from -0.051 and 0.039 $mg.mg^{-1}$ so we could be 95% confident that the difference in the sample of chlorophyll estimation was expected to be between -0.051 and 0.039 $mg.mg^{-1}$. The confidence limit (upper limit - lower limit of agreement) of chlorophyll estimation by BAVI was 0.09 mg.mg-1, which equals to 8.28% of the established method (SPAD-502). Therefore, we could say 95% confidently that estimation of chlorophyll made by BAVI method would be 4.14% (half of the confidence limit) higher or lower than SPAD estimation. Meanwhile, the limit of agreement between NSPAD and NBAVI was from -0.092 to 0.081%, so the confidence limit was 0.173% which equals to 5.46% of the mean SPAD-502 estimation. It could be said 95% confidently, then, that the foliar nitrogen content estimated by BAVI method would have a maximum difference of ±2.73% than SPAD-502 estimation.
Given that the difference between the two compared methods was normally distributed, the precision of the bias could be computed with the 95% confidence interval (CI) (Hanneman, 2008). As presented in Table 2, the 95% CI for foliar chlorophyll measurement bias was -0.017 to 0.006 $mg.mg^{-1}$ while for N content measurement was -0.027 to 0.017%. Therefore, we could be 95% confident that the true bias of the population of chlorophyll estimation would fall between -0.017 and 0.006 $mg.mg^{-1}$, while the true bias of N estimations would fall between -0.027 to 0.017%.
The result of the comparations showed that BAVI is a promising method to estimates tea shoots chlorophyll and nitrogen content. While it may produce biases up to ±4.14% when compared to chlorophyll content and ±2.73% than nitrogen content estimated by SPAD-502, BAVI derived from aerial images captured from UAV offer some significant benefits. BAVI provides a very fast estimation as a UAV may capture hundreds of hectares of aerial images in minutes. It is possible to estimates foliar chlorophyll or nitrogen content of all of the plants covered by aerial images without having to interpolate as measured with SPAD-502. It must be noted that interpolation of chlorophyll or nitrogen content measurements samples to estimate other plants' chlorophyll or nitrogen content would generate errors. Indeed, it was a question whether the error of interpolation is higher or lower than the bias come with BAVI estimation.

CONCLUSION

A radically new approach to develop foliar chlorophyll and nitrogen content of crops measurement has been proposed. The key idea is to measure all of plant, not just some samples, as fast and as low cost as possible. In this way, it will improve crop production management which enable planters to treat their plants precisely, individually. This will enable the assessment of the quality of tea harvests quickly and cheaply and help producers manage the quality of their production.

The results showed that B*A* vegetation index derived from uncalibrated aerial image captured from UAV can be used to estimate tea shoots chlorophyll and nitrogen content instead of SPAD-502. The maximum bias of the measurement, with 95% confident, was 4.14% (foliar chlorophyll content measurement) and 2.73% (foliar nitrogen content measurement) of the SPAD-502 method.

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