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Evaluation of the Use of SPAD-502 Chlorophyll meter for non-destructive estimation of nitrogen status of tea leaf (*Camellia sinensis* L. Kuntze)

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ABSTRACT

Efficient nutrient management requires estimating factual fertilizer requirements. This study was aimed to test the use of chlorophyll meter SPAD-502 to estimate the nitrogen status of tea maintenance leaf. The test was carried out by correlating the SPAD readings with destructively measured leaf nitrogen content using samples oGbtained from nitrogen fertilizer dosage experiments. Observations were made at 15, 32, 45 and 62 days after the application of N fertilizer treatments. The results showed that the SPAD readings and total nitrogen leaf content correlated significantly with the time of observation. Estimation of leaf N content based on the SPAD readings follows linear line equation $y = 0.0311x + 1.5856$ with coefficient determinant (R^2) = 0.62 significantly at $P<0.01$. It was concluded that SPAD-502 chlorophyll meter is reliable to assess the leaf nitrogen content of tea maintenance leaf and is adequate to predict future nitrogen fertilizer requirements.

Key words: *Camellia sinensis* L., Foliar nitrogen, Non-destructive, SPAD-502.

INTRODUCTION

Tea plantations in Indonesia generally harvest throughout the year continously (Anonymous, 2017). To cope with nutrients that are drained through harvesting, they usually fertilize 3-4 times per year or every 3-4 month (Effendi *et al.*, 2011). Among other nutrients, nitrogen is the most important that affects the quality and growth of tea plants since the nursery to crops (Mudau *et al.*, 2007). It is one of the important reasons why nitrogen availability as a determinant of productivity (Sarwar *et al.*, 2007). However, excessive N nutrients in the soil can have negative effects on tea growth and production (Okano *et al.*, 1997). Tea plantations, which are affected by clones and the age of plants, received excessive N fertilizing had their tea production decreased, as reported in several plantations in India (Dutta, 2011). The rate of N fertilizer application, therefore, must be as close as possible with the factual needs of plants to achieve optimum growth, production, and quality which could not be provided by the soil. The challenge is how to get the best estimate of appropriate N fertilizer application rate. It could be very complicated task as soil nitrogen availability is affected by many factors such as weather, soil microbial activity, and leaching process. The availability of a method capable to easily and rapidly provide not only the amount of fertilizer needs estimate but also its future prediction and when it should be applied, would be valuable in managing tea plantation.

In addition to the soil N measurements which provide its availability Fig, N nutrient status is also approached by measuring foliar N content both destructively and non-destructively. Destructive measurement of plant leaf N content is done by tissue analysis. The plant tissue analysis procedure involves the collection of samples, washing, drying, preparation, and laboratory analysis which is cost and time consuming. The results of the measurement of destructive methods are generally only useful for the next planting season (Muñoz-Huerta *et al.*, 2013).

Chlorophyll meter SPAD-502 is a portable device that allows non-destructive assessment of leaf chlorophyll content easily and rapidly. It emits light actively at of 650 nm (red) and 940 nm (near infrared) wavelength to the leaf samples and measures its transmittance (Patane and Vibhute, 2014). The transmittance value of both wavelength are then converted into one value which is called the SPAD reading value. The SPAD value is known to correlate closely with chlorophyll content (Coste *et al.*, 2010), thus it can be theoretically linked to leaf nitrogen content or plant yield due to close relationship between leaf chlorophyll content and leaf nitrogen content and yield. SPAD has been used to help to estimate nitrogen status of chickpea (Mudalagiriyappa *et al.*, 2016), rice (Pushpanathan *et al.*, 2004), and maize (Hokmalipour and Darbandi, 2011). It was reported that implemention of SPAD based site specific nitrogen

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management reduced a half number of farmer fertilizing practice to achieve higher productifity of rice (Yadav *et al.*, 2017). Calibration of the value of SPAD readings to the leaf nitrogen content of different types of plants is needed before it can be used to N application rate estimation. Investigation on correlation of SPAD readings with certain plant type will be useful to develop a method capable to estimate the most efficient rate of N application. The easiness, portability, and cost effectiveness of SPAD makes it helpful for the planters to implement site specific approach on their plantations.

This study was aimed to evaluate the use of SPAD-502 chlorophyll meter in determining the nitrogen content of maintenance leaf of tea plants and wether it can be used to predict future N status of tea leaf.

MATERIALS AND METHODS

The experiment was conducted from February untill May 2015 at PTPN XII Wonosari Tea Plantation, Lawang, Kabupaten Malang, Jawa Timur, Indonesia. The plantation is geographically located at -07°49'17.6" (latitude); 112°38'36" (longitude). It is 905-1050 meters above sea level which dominated with Andosol and annual rainfall approximately 3000 mm.

Data were acquired from an N dose experiment comprised of 6 levels of treatment with 3 replications. Each of plots was 18 x 27 meters^2 which selected from a homogeneous 3 years old after pruning productive crop and arranged in a completely randomized design. Treatment levels of nitrogen dose was determined based on the percentage of the highest dose applied by the company at the trial location, namely: 0 (control), 25, 50, 75, 100 and 125% with 3 replications. The highest dose of reference used was 350 Kg N.Ha^{-1}.year^{-1} (Effendi *et al.*, 2011). Referring to the practice of fertilization at the research site, which is done 4 times a year, then the dose of applied fertilizer was a quarter of the recommended dose. 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 2 weeks before the first observation, after picking time by burrying it in 4 separate points, each of which was 60 cm from the stem.

Observations were carried out 4 times at 15, 32, 45, and 62 days after treatment (DAT). Leaf samples were maintenance leaves taken from 6 randomly selected locations of each experimental plot. Measurements with chlorophyll meter were carried out on each sample leaf. Measurements were made 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). SPAD readings were performed on each leaf face so that 6 observations were obtained which were then averaged to get the 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 sample leaves were then analyzed directly or frozen at -20^{o}C until it was analyzed. The leaves were dried at 105^{o}C for 30 minutes to quickly stop polyphenol oxidase enzyme activities and then at 70^{o}C until a stable dry weight was reached (Wang *et al.*, 2017). Dried leaf was, then, crushed and the N content was calculated using the Kjehldahl method. Measurements were carried out 3 times each and then averaged as a value of 1 sample.

The central tendency of collected data of each variable was measured by mean and median, while the variability was measured using variance, standard deviation, coefficient of variation (CV), and range. Evaluation of CV of the data was based on 3 categories, namely: low (less than 15%), medium (15-35%), and high (>35%) (Gholizadeh *et al.*, 2017). Correlation between variables were tested by 2 tail Pearson Correlation Test with error probability less than 5% ($P<0.05$). Whenever significant correlation noted, the relationships between the variables were defined by regression analysis. All of statistical analysis were performed using IBM SPSS Statistics for Windows, Version 23 (IBM Corp., 2015).

RESULTS AND DISCUSSION

Descriptive statistics of foliar N content and SPAD readings from 15, 32, 45 and 62 DAT are presented at Table 1. The CV of the SPAD readings from 15, 32, 45 and 62 DAT observations were 14.02, 11.29, 6.19 and 9.66% respectively. Those variability of non-destructively measured

Table 1: Descriptive statistic of N content and SPAD readings of tea maintenance leaves.

	Foliar N Content (%)				SPAD readings			
Parameters	**15 DAT**	**32 DAT**	**45 DAT**	**62 DAT**	**15 DAT**	**32 DAT**	**45 DAT**	**62 DAT**
Mean	3.18	3.62	3.65	3.46	56.22	62.42	65.05	60.78
Median	3.18	3.60	3.69	3.48	55.06	62.74	66.15	60.80
Standard Dev.	0.124	0.163	0.184	0.204	7.88	7.05	4.03	5.87
Variance	0.015	0.026	0.034	0.042	62.09	49.64	16.24	34.46
CV (%)	3.92	4.50	5.05	5.89	14.02	11.29	6.19	9.66
Minimum	3.02	3.22	3.13	3.07	45.77	50.17	55.38	50.03
Maximum	3.43	3.85	3.85	3.77	72.15	71.88	71.60	75.52
Range	0.42	0.63	0.72	0.70	26.38	21.72	16.22	25.48

DAT: days after treatment .

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Table 2: Correlation coefficients of SPAD readings and total N content of maintenance leaves of tea at different observations.

	15 DAT	32 DAT	45 DAT	62 DAT
Correlation coeffient	0.82*	0.96**	0.99**	0.94**

* (P<0.05); ** (P<0.01)

values were generally higher than the variability of destructive N measurements which were 3.92, 4.50, 5.05 and 5.89% of the consecutive observations. The lower but not significant SPAD CV compared to the measurement of destructive N leaf content only occurred at 45 days after treatment. This indicates that the data homogeneity with destructive methods is higher, or it can be said that non-destructive measurements using SPAD produce more heterogeneous data. Based on the criteria previously described, the variability of both measurements is categorized as low so that the data can be said to be quite homogeneous.

The N dosage treatment significantly affects foliar N content and SPAD reading at 45 DAT observation as presented at Fig 1 (A) and Fig 1 (B) respectively. This finding confirm other previous studies results different crops such as turfgrass (Rodriguez and Miller, 2000), potato (Güler, 2009), corn (Rashid *et al.*, 2005; Varvel *et al.*, 1997). The best fit regression model to describe the effect of N dosage on both variables is quadratic polynomial. Regression equation describing the effects of N dosage on foliar N content is $y = -4E\text{-}05x^2 + 0.0065x + 3.4899$ with $R^2 = 0.88$ significantly at $P<0.01$, while on SPAD reading is $y = -0.0016x^2 + 0.2386x + 58.848$ with $R^2 = 0.95$ significantly at $P<0.01$. Pearson Correlation Test results presented at Table 2 show significant correlations between SPAD readings and maintenance leaves N content at all observations ($P<0,05$). These explains the similarity of regression line patterns of both variables.

Temporal observation shows the pattern similarity between the mean of N leaf content and SPAD reading, there is an increase up to 45 DAT followed by a decrease in 62 DAT (Table 1). These indicate the pattern of N absorbed by the plants from time to time after the fertilization treatment. Pearson correlation test showed a correlation coefficient of 0.64 ($P<0.01$) between the measurement time with the SPAD

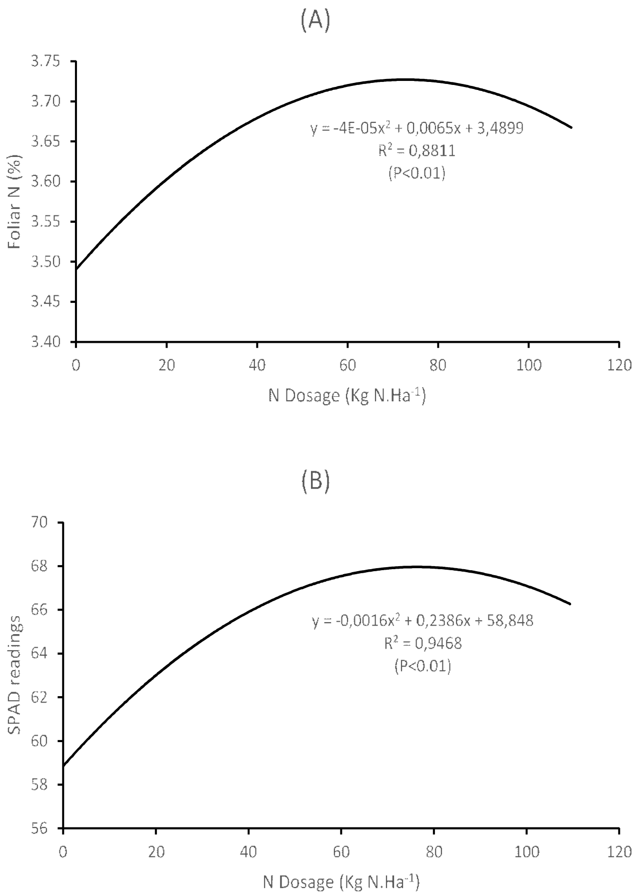

Fig 1: Effects of N dosage on maintenance leaves N content (A) and SPAD readings (B).

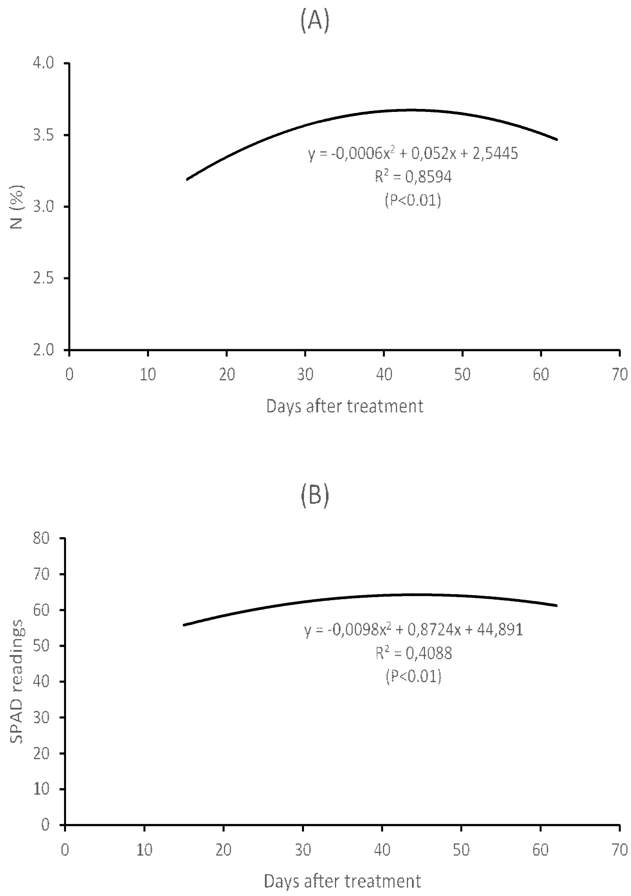

Fig 2: Effects of time after N fertilizer application on maintenance leaf N content (A) and SPAD readings (B).

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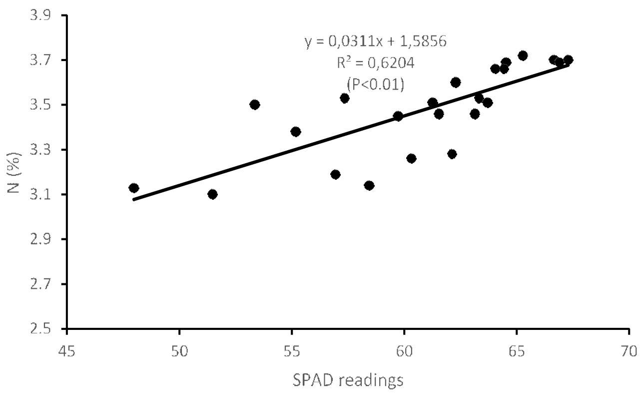

Fig 3: Relationship between SPAD readings and maintenance tea leaf N content.

reading, while between the measurement time with the N leaf content it showed of 0.92 (P <0.01). The significant correlations between time of observation and SPAD reading and between SPAD reading and foliar N content indicate that SPAD readings can be used to predict future foliar N content. The capability to predict future foliar N content is useful for planters to properly respond to the need of N fertilizer. It provides a good basis to make necessary adjustment on fertilizing plan.

Regression analysis showed that the best fit model to describe the effect of time on foliar N content and SPAD readings are quadratic polynomial as shown in Fig 2A and 2B. The relationship of time after applying N fertilizer with SPAD readings follows the equation $y = -0.0098x^2 + 0.8724x + 44.8891$ with $R^2 = 0.41$ ($P<0.01$), while the relationship of time with foliar N content follows the equation $y = -0.0006x^2 + 0.052x + 2.5445$ with $R^2 = 0.86$ ($P<0.01$). However, the pattern will be influenced by the method and frequency of harvesting of tea leaves. In this study harvesting was done manually with total shoot removal. The more intensive harvesting of tea leaves will cause the soil nitrogen to drain more quickly, thus affecting the availability of N afterwards.

Pearson correlation test (2-tail) on SPAD readings and foliar N content shows significant coefficients ($P<0.05$) in all observations (Table 2). These findings indicate that all observations of SPAD readings can be used as an estimator of maintenance leaves N content. These finding is consistent with other previous studies' findings (Brunetto *et al.*, 2012; Patane and Vibhute, 2014; Xiong *et al.*, 2015). Correlation test between SPAD readings and foliar N content from all observations resulted in a significant positive correlation coefficient ($r = 0.79$; $P<0.01$), as expected based on significant correlation of each observation. Regression analysis to explain the relationship between SPAD readings and foliar N content using all observations data is presented in Fig 3.

The relationship between these two variables follows the linear equation $y = 0.0311x + 1.5856$ with $R^2 = 0.62$ significantly at $P<0.01$. These results suggest that SPAD readings may be used as a rapid method of assessing tea plant nitrogen status, a factual basis for estimation of nitrogen fertilizer application rate.

CONCLUSION

SPAD reading was found to be strongly correlated with the N leaf content of tea maintenance so that it could be used to estimate the status of N tea nutrients. These findings indicate that the method of estimating non-destructive tea leaf nitrogen content using chlorophyll meter SPAD-502 has the potential to improve the efficiency of N fertilization on tea plantations. Furthermore, the results of non-destructive assessment of the N status of the tea plant can be used to improve the efficiency of N fertilization. Data analysis on the results of observations between times can be used to predict future N levels and N needs. Further research is needed to examine the application of the estimation model of different research locations and seasons.

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