1 **Development of non-destructive methods to estimate functional traits and**
2 **field evaluation in tea plantations using a smartphone**

3 **Esack Edwin Raj*, Rajagobal Raj Kumar, Ashokraj Shanmugam and Balakrishnan**
4 **Radhakrishnan**

5 Plant Physiology and Biotechnology Division, UPASI Tea Research Foundation, Tea Research
6 Institute, Nirar Dam PO, Valparai 642 127, Tamil Nadu, INDIA.

7 *E-mail: edwinrht@gmail.com

8 Abstract

9 Smartphones are equipped with various types of sensors which make them a promising tool
 10 to assist diverse digital farming tasks because of their mobility, cost, accessibility, and
 11 computing power allow us to perform real-time practical applications. This paper presents
 12 the utilization of various non-destructive methods of nutrient and disease classification
 13 techniques using smartphone collected images, processed through various image
 14 segmentation algorithms. Both *in vivo* and *in vitro* estimations shows comparable results
 15 with both chlorophyll and nitrogen contents of a crop shoot. Moreover, the correlation
 16 between SPAD measured values and nitrogen of crop shoot showed a significant linear
 17 association ($R^2=0.7309$), revealing the potency of *in vivo* observation for prediction of actual
 18 chlorophyll content in tea crop. SPAD values and yield have a strong linear relationship
 19 ($R^2=0.7103$), in which SPAD-meter performed better detection at very low values. The study
 20 concluded that the proposed techniques could be used for automatic detection as well as
 21 classification of foliar diseases and nutrients in tea.

22 **Keywords:** Chlorophyll; Disease assessment; Image segmentation; Nitrogen; SPAD meter.

23 Introduction

24 In the technology era, industries are shifting from manual to automated solutions for
 25 various problems. The developed technology has not only augmented the efficiency, but
 26 they also have shortened the time, cost, and labour required to get assured excellence. In
 27 agriculture, tea is one of the major economic plantation crops casing a large amount of area
 28 and labour. Presently, the industry as a whole facing lot of problems in which nutrient and
 29 disease management are primary concerns besides labour shortage, in turn, leads to
 30 economic volatility. The idea behind this research is to develop precision non-destructive
 31 methods which can work out for the problem of nutrient and disease assessment in the tea
 32 crop by examining the image. In the modern world, image processing has fetched it in
 33 reality which can be used in any ecological conditions and lead to very momentous, reliable
 34 and precise elucidations in problem-solving. Such a study can give a clear idea about
 35 nutrition status and its kind, the area of pest incidences, pesticide requirement or just to do
 36 what is necessary, etc., for a crop by comparing the colour of a plant's leaves with the
 37 endorsements given by agricultural organizations. The tea industry is highly dependent on
 38 productivity where potentially destructive blight diseases can result in ~35% crop loss.
 39 Therefore, the use of disease detection technique plays an important role that could enable
 40 planters to take preventive/quarantine measures at the initial stage of disease transition.

41 Chlorophylls play an important role in the plant as primary photosynthetic pigment
 42 composed together with carotenoids exhibits a unique colour appearance which is specific
 43 to genotype and even used as a parameter of physiological maturity, nutrient status and
 44 quality (Pagola et al. 2009). The chlorophyll content is strongly related to the colour and
 45 flavour of the tea is principally determined by crop shoot chemical constituents (Wang et al.,
 46 2004) and is positively correlated with the scores for appearance and infused leaf (Wang et
 47 al., 2014). Leaf chlorophyll is mostly used as an index to diagnose diseases and nitrogen (N)
 48 status in the plants besides minor nutrient symptoms. N is the most required mineral
 49 nutrient in tea where the optimum and timely application is crucial in achieving a higher
 50 yield. However, an excess application of N in the agricultural environment can lead to water
 51 pollution and upon intake, it is transformed into nitrosamines molecules acting as
 52 carcinogens (Turner and Rabalais, 1991). Hence, site-specific timely application of N at

53 required quantity with a spatial concern could be a sustainable method to overcome the
54 limitations of traditional replacement theory; however, methods have to be developed for
55 real-time estimation along with cost-effectiveness.

56 Although disease symptom and nutrient status of the plant can be assessed by
57 human observation, yet initial stage identification, cause for a larger area and their spatial
58 relationship with an associated environmental variable are quite impossible for a common
59 agrarian but very important for disease dynamics. So far, various conventional invasive
60 methods have been practised and modern non-destructive methods are being developed to
61 assess the leaf chlorophyll, nitrogen and disease of various crop plants. Conventional
62 methods of determining the chlorophyll and nitrogen content are destructive
63 chromatographic methods, laborious and are not adaptable to real-time estimation
64 (Gilmore and Yamamoto, 1991). As an alternative, the non-destructive image processing
65 technique is an emerging field of agriculture, enabling real-time measurement using digital
66 images to get the intrinsic characteristics of leaf i.e. colour and texture and are related to
67 geographical information. This method is a very easy, portable, less time consuming, cost-
68 effective and provides accurate information and understanding at a spatial scale. The
69 proposed image processing method in the study deals with trichromatic colours, i.e. RGB
70 and compared to the destructive methods for nitrogen and chlorophyll contents besides
71 disease detection by using various statistical analyses.

72 **Material and methods**

73 The overview of the methodology proposed in the study depicted in Fig.-1. Initially, the
74 chlorophyll content of UPASI clones was chemically estimated at different seasons for two
75 agricultural years using acetone after recording SPAD-502 chlorophyll meter (Konica Minolta
76 Sensing Inc., Tokyo, Japan). The same leaves were photographed using a smartphone for the
77 estimation of chlorophyll using image processing algorithms. The settings of the camera,
78 includes enabling GPS, sensor light sensitivity (ISO) and exposure time were set in auto
79 mode and the camera could select them based on light conditions. The histogram of leaf
80 image was obtained using ImageJ and extracted RGB values correlated with the SPAD and
81 chemically estimated chlorophyll. Neural network analysis with genetic algorithm was used

82 to estimate the plant pigments by training (70% of the data) and validation (30% of the
83 data). The accuracy of the developed model is based on R^2 , SE and RMSE.

84 For the development of a non-destructive method of N estimation, a randomised
85 completely block designed (RCBD) experiment was conducted with UPASI-9 clone after two
86 years of pruning. Each treatment replicated three times and each block consisted of ~100
87 plants. Experimental plots treated with various levels of Nitrogen (0, 150, 300, 600, 900 and
88 1600 kg/ha/y in four splits) and images were taken before and after every harvest. Mother
89 leaf collected from respective plots subjected to shade drying and grinding, the N content of
90 the samples was determined by Kjeldahl digestion technique.

91 Image segmentation algorithm used to classify the diseased with non-diseased parts
92 in the leaf using neural networks with a genetic algorithm. In the first step of fitness
93 computation, the dataset of the pixel is clustered according to nearest respective cluster
94 centres such that each pixel X_i of the colour image is put into the respective cluster with the
95 cluster centre z_j for $j = 1, 2, \dots, K$ by the following equations

$$96 \quad \text{If } \|X_i - Z_j\| < \|X_i - Z_l\|$$

$$i = 1, 2, \dots, m \times n, \quad l = 1, 2, \dots, K \text{ and } p \neq j$$

97 The percentage of disease was spatially interpolated geo-statistically using the kriging
98 method in which the weights are assessed by the autocorrelation to obtain unbiased error
99 and minimum estimation variance. Kriging interpolation uses the attribute values (i.e.
100 nugget, sill, and range values) obtained from semivariogram analysis and was evaluated
101 through a leave-one-out cross-validation approach (Gholizadeh et al., 2012). Maps were
102 created in ArcGIS software.

103 **Results and Discussion**

104 **Estimation of chlorophyll**

105 Initially, acetone extracted leaf pigments were estimated and the results are correlated with
106 the RGB features extracted from smartphone capture images using ImageJ software was
107 shown in Fig.-2. The correlation between the SPAD estimated pigment values and image
108 processing values shown in Fig.-3. A significant and positive correlation was observed

between SPAD meter reading and image algorithm reading at each stage of measurement. The coefficients of determination (R^2) indicated that there was a minor variation in chlorophyll content which was explained by image processing algorithm reading at each measurement stage of development (Fig.-3b) that ranging from 0.8 to 2% (Table-1). The relationship between image feature and SPAD values indicated that red ($R^2 = 0.830$) and green ($R^2 = 0.857$) colours are highly related with the SPAD values except for blue colour ($R^2 = 0.006$). The result also indicated the SPAD values are negatively correlated with the red ($y = -3.650x + 302.054$) and green ($y = -2.435x + 278.679$) while blue is positively related ($y = 0.107x + 13.194$; Fig.-3a). The results show that the image processing algorithm readings can be used as an effective indicator for evaluating the chlorophyll content of the leaf at a different stage of the crop. Based on this exercise, the reader must conclude that surface chlorophyll measurements are worthwhile by making use of an image processing algorithm.

Measuring the nitrogen status and its relationship with yield

A regression analysis was made between SPAD readings and leaf N content besides the yield. There was a significant positive linear relationship between both the attributes as displayed in Fig.-4. Regression analysis of the SPAD readings and yield demonstrated that these attributes produced a significant linear relationship $R^2 = 0.7309$; $y = 85.213x - 3980.2$ (Fig.-4a). Similarly, the regression analysis of the SPAD readings and N demonstrated a significant linear relationship $R^2 = 0.7103$; $y = 0.1868x - 7.8051$ (Fig.-4b). Similar results were arrived by Yang et al. (2014), Ramesh et al. (2002) and Swain & Sandip (2010) who established the linear relationships between leaf N content and SPAD readings and highly significant correlations of SPAD readings with the yield in various crops.

Clonal Characterisation

The clonal signature of the UPASI released clones and an important estate selection was created to identifying the superior materials from the seedling blocks or infillings of an area of interest. The signature of the respective clones was established using a supervised image classification method and the algorithm was applied to the images of seedling fields. The plotrix of the UPASI released clones was depicted in the Fig.-5. From Fig.-6, it was observed that using supervised image segmentation algorithm can be exploited for the identification

and composition of infilling clones in the area or some of the elite clones like materials from the seedlings block for crop improvement.

Disease assessment by Image segmentation

For the assessment of blister blight disease, the images were subjected to K-mean hierarchical clustering and the area of infected portions was measured from the targeted cluster. Fig. 7 shows the original images followed by output segmented clusters revealed that the captured image can be classified into a different class of diseases. The classification of images with the Minimum Distance Criterion shows that there was about 95% accuracy in measuring the area of the target and the remaining 5% error consider as misclassification. Form Fig.-8, it was understood that inbuilt GPS of a smartphone can be utilised for plotting the disease occurrence with geographic information. This method can be used for monitoring disease dynamics before and after the application fungicides on a real-time basis.

Conclusion

The study demonstrated various non-invasive image processing methods for the quantification of chlorophyll, nitrogen and disease. The methods can enable common agrarian for real-time estimation and assessment of nutrients and disease and its spatial dynamics for larger area management. Further study is required for validation and developing an Android application for planter's utility.

Reference

- Gholizadeh A, Amin MSM, Anuar AR, AimrunW. Relationship between apparent electrical conductivity and soil physical properties in a Malaysian paddy field. Arch Agron Soil Sci 2012;58:155–68.
- Gilmore, A. M. and Yamamoto, H.Y. 1991. Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C-18 high-performance liquid chromatographic column. J. Chromatography A. 543: 137-145.

164 Pagola, M., R. Ortiz, I. Irigoyen, H. Bustince, E. Barrenechea, P. Apariciotejo, C. Lamsfus, and
165 B. Lasa, 2008. New method to assess barley nitrogen nutrition status based on image
166 colour analysis Comparison with SPAD-502. *Comput. Electron. Agric.*, **5**, 213–218.

167 Ramesh K, Chandrasekaran B, Balasubramanian TN, Bangarusamy U, Sivasamy R, Sankaran
168 N. 2002. Chlorophyll dynamics in rice (*Oryza sativa*) before and after flowering based
169 on SPAD (chlorophyll) meter monitoring and its relation with grain yield. *J Agron Crop*
170 *Sci*; 188:102–5.

171 Swain DK, Sandip SJ. 2010. Development of SPAD values of medium-and long-duration rice
172 variety for site-specific nitrogen management. *J Agron* 9:38–44.

173 Turner, R.E., Rabalais, N.N., 1991. Changes in Mississippi River water quality this century.
174 *Bioscience* 41, 140–147.

175 Wang Y, Wang D, Shi P, Omasa K. 2014. Estimating rice chlorophyll content and leaf nitrogen
176 concentration with a digital still colour camera under natural light. *Plant Methods*
177 10:36.

178 Wang, Q., J. Chen, and Y. Li. 2004. Non-destructive and rapid estimation of leaf chlorophyll
179 and nitrogen status of peace lily using a chlorophyll meter. *Journal of plant nutrition*
180 27, 557-569.

181 Yang H, Li J, Yang J, Wang H, Zou J. 2014. Effects of nitrogen application rate and leaf age on
182 the distribution pattern of leaf SPAD readings in the rice canopy. *PLoS ONE*;9: e88421.

183

Fig.-1: Schematic overview of the proposed study

Fig.-2: The relationship between RGB features and chemically estimated plant pigments

Fig.-3: Relationship between SPAD values and chemically estimated values at various stages (A & B). Validation of estimated plant pigments between chemically estimated and image extracted chlorophyll-a (C), similarly with SPAD estimated chlorophyll-a (D).

Fig.-4: Relationship between SPAD values and yield (A); and SPAD with Nitrogen (B)

UPASI-1	UPASI-2	UPASI-3	UPASI-4	UPASI-5	UPASI-6	UPASI-7	UPASI-8	UPASI-9	UPASI-10
UPASI-11	UPASI-12	UPASI-13	UPASI-14	UPASI-15	UPASI-16	UPASI-17	UPASI-18	UPASI-19	UPASI-20
UPASI-21	UPASI-22	UPASI-23	UPASI-24	UPASI-25	UPASI-26	UPASI-27	UPASI-28	TRF-1	TRF-2
TRF-3	TRF-4	TRF-5	TRI-2024	TRI-2025	TRI-2043	SA-6	SMP-1	TES-34	ATK-1

Fig.-5: Colour profile of UPASI clones and estate selections

Fig.-6: Application of supervised classification method for clonal identification (top row) and elite clones like materials in the seedling block (bottom row) using image segmentation analysis.

Fig.-7: Application of image processing methods for disease assessment

Fig.-8: Spatial interpolation of blister disease (A) percentage before (B) and after chemical control (C).

Table-1: Validation of chemically estimated pigments with the SPAD and image extracted values

SPAD	R ²	SE	RMSE	Image	R ²	SE	RMSE
Chl-a	0.862	0.322	0.568	Chl-a	0.951	0.061	0.247
Chl-b	0.761	0.233	0.482	Chl-b	0.968	0.027	0.165
Caro	0.997	0.014	0.120	Caro	0.931	0.024	0.156
T-Chl	0.946	0.280	0.529	TChl	0.938	0.101	0.317
Xantho	0.666	0.095	0.308	Xantho	0.974	0.007	0.086

Chl-a: Chlorophyll-a; Chl-b: Chlorophyll-b; Caro: Carotenoid; T-Chl; Total Chlorophyll; Xantho: Xanthophyll; the unit of all the plant pigments are expressed as mg/g; R²: Correlation coefficient values; SE: standard error; and RMSE: Root mean sum square error.