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Research Article

ESTIMATION OF CHLOROPHYLL USING IMAGE PROCESSING

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ABSTRACT

In this study, we present a modest and easily operatable technique that determines the chlorophyll content of plant using an Image Processing Technique. Chlorophyll provides the nutrient status of a plant and nitrogen is a radial part of the chlorophyll, which is the primary absorber of light energy needed for photosynthesis. Quality of plant is determined by the green color of plants which is achieved through Chlorophyll and nitrogen. Plants with high chlorophyll content are green and healthy, whereas plants with less chlorophyll content seems to be a pale green or yellow in color and remain small and retarded. Thus leaf color can be used to determine chlorophyll content by using Image Processing techniques.

At present chlorophyll content measurement is usually done through methods such as Spectrophotometer (destructive method), SPAD, at Leaf (Non-destructive). But these methods are expensive, time-consuming and unavailable to remote rural farmers, and various other such issues.

We propose to study and develop an expert based system that will use Image Processing to determine the Chlorophyll content of a plant through image acquired by a smart phone.

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INTRODUCTION

Usually plants require 3 major chemicals NPK (i.e. Nitrogen, Phosphorous and Potassium). Among them, Nitrogen is one of the most important nutrient necessary for healthy plant growth and development. Nitrogen is not directly available from atmosphere and earth crust and hence nitrogen deficiency is the most common problem that affects a plant. Nitrogen is the vital one because it is a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from Water and Carbondioxide (i.e. Photosynthesis). Whereas, Phosphorous is involved in the metabolic processes responsible for transferring energy from one point to another in a plant. And, Potassium helps to regulate plant metabolism.

Therefore, Leaf color is a good indicator of plant health and nutrition status. The green color of the plant canopies is associated with leaf chlorophyll content, which is positively correlated with leaf nitrogen, because nitrogen is a part of chlorophyll molecule, which gives plants their green color and is involved in creating food for plant through photosynthesis.

Although there are laboratory techniques that can quantify plant nitrogen, these can be time consuming and cost-prohibitive to perform on a regular basis.

Nitrogen content in the leaves can also be determined by analyzing the distributed of color components ((R) Red, (G) Green, (B) Blue and Hue (H), Saturation (S) and Intensity (I)) of the image of a single leaf or group of plants. Determining of the chlorophyll content of the plants gives valuable information relevant to plant health and crop management.

The prior objective of this study is to develop an expert based system to determine nutrient status of plants using an algorithm to estimate the chlorophyll content in leaves with a smartphones camera and Image Processing Technique.

Recent study

The literature survey has been done to get deep information about the estimation of chlorophyll content in plant using different techniques so far.

In the study of Farshad Vesali et al. [1], an Android App(SmartSPAD) for smartphone was developed to estimate the chlorophyll content of corn leaves was based on contact

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imaging. For SPAD (Soil Plant Analysis Development) values estimation, the selected features were used as inputs to the linear (regression) and neural network models and R² and RMSE values for the linear model were 0.74 and 6.2 and for the neural network model were 0.82 and 5.10, respectively. The estimated Smart SPAD values were compared well with the corresponding SPAD meter values (R² =0.88 and 0.72, and RMSE = 4.03 and 5.96 for neural network and linear model, respectively).

In the study of Mahmood Mahmoodi *et al.*[2], a chemical and SPAD methods was used to estimate the chlorophyll content of leaves of five walnut varieties and the Chl. content values were compared with color features of leaves to determine the correlation between Chl. content and leaf color features. To determine the color features, the color components of red (R), green (G) and blue (B) in RGB space and hue (H), saturation (S) and intensity (I) in HSI space were determined and (R-B)/(R+B) function was used for analysis. The analyses showed that there were correlation coefficients of -0.78 and 0.87 between (R-B)/(R+B) and hue with Chl. content, respectively and hue color component had the most correlation coefficient with Chl. content among the analyses.

In the study of M. M. Ali *et al.* [3], plant Chl. and N levels using the RGB (Red, Green and Blue) color model was developed. The value of Chl. and N was measured in laboratory and compared with the nondestructive methods of SPAD 502 and Dark Green Color Index (DGCI) and R = 0.96 value for chl. and R=0.85 value for N was achieved to outperform both SPAD and DGCI.

In the study of M. Kokila *et al.* [4], the chlorophyll content of maize leaf was estimated using an image capturing technique (i.e. Unmanned Aerial Vehicle, UAV) and it is segmented to cleave the green pixel. They used various algorithm ((R-B)/(R+B), G/R, R/(R+G+B), R+G and (255/R+B)-(255/R-G)/3) to get a better correlated result with laboratory measured chlorophyll content of the plant and correlation value of -0.84 in (255/R+B) - (255/R-B)/3 gave the better result.

In the study of Shigeto Kawashima *et al.*[5], chlorophyll content of leaves was estimated using a portable color video camera and a personal computer. Anormalized difference between chlorophyll content and various function of RGB values i.e. (R-B)/(R+B) for which the slope of the regression line is -1.76±0.09 (i.e.=5%) and the intercept 0.952±0.027 (i.e.=3%) and the root mean square of residuals is 0.10 gm⁻².

In the study of P. Karthika *et al.*[6],Chlorophyll content was estimated in three types of papaya leaf(i.e. dry leaf, Tender leaf, healthy leaf) using mathematical operations Chl.= (R+B)/255 - (R-B)/255 /3 and superior results was achieved with comparison to the true value of chlorophyll content measured in the laboratory and existing Chl methods.

In the study Satya Prakash Yadav *et al.*[7], non-invasive determination of chlorophyll content of leaves of micro propagated potato plants using RGB based image analysis was developed by using the trichromatic colors, where R and G negatively correlated with the chlorophyll content, while a positive correlation was observed with B chromate. The use of mean brightness ratio improved the relationship of the tricolors with chlorophyll content. Spectral properties such as luminosity

and saturation were also found to be negatively correlated with the chlorophyll content.

Proposed Methodology

Plant material and Image Acquisition

Collect the sample of leaves. Capture the image of collected samples using contact imaging (LASAP) technique with a constant light of 10-12 lumens. Chlorophyll content of leaves collected is calculated from laboratory using manual process which becomes the reference for development of proposed system here we are using.

Proposed System

Plant: Assam Lemon (*Citrus Limon L. Burmf*) was collected from Horticulture Research Station Assam Agriculture University, Kahikuchi, Guwahati, Assam. And the plant leaves were divided into three types, Tender leaf, immature leaf and mature leaf.

Image Acquisition

For image capturing, a **Lenovo Z2 Plus** smart phone with CCD sensor camera and a constant light source i.e. Eveready Digital Torch (0.2 watt, 11 lumen) was used. In this method, leaves are held to the camera lens of the smart phone and the camera captures the light passing through the leaf. Compared to other standards of image capturing, the method of contact imaging has several advantages including:

1. There is no interference from the background.
2. There is no variation in the distance between leaf and sensor.
3. There are no differences in image focus or blur.
4. Lower influence of different ambient conditions.
5. Minimal effects of camera-to-camera variation.
6. No need to remove the back ground and the entire image can be used as an input data.

Data Collection

Extraction of chlorophyll by spectrophotometer (Arnon, 1949)

One gram of finely cut fresh leaves were taken and ground with 20 – 40ml of 80% acetone. It was then centrifuged at 5000 – 10000rpm for 5mins. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The absorbance of the solution was read at 645nm and 663nm against the solvent (acetone) blank.

Estimation of Chlorophyll content

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Total Chlorophyll: 20.2(A645) + 8.02(A663)

Chlorophyll a: 12.7(A663) – 2.69(A645)

Chlorophyll b: 22.9(A645) – 4.68(A663)

Feature Extraction

Feature extraction includes color image processing steps to measure the green color purity to indicate the amount of chlorophyll present in the leaf.

For calculating the mean rgb values of leaves MATLAB version 2015 is used with the following steps.

Step1:- Image are captured using a smartphone and loaded into MATLAB.

Step2:- Images are Enhanced to improve their quality.

Step3:- Mean r, Mean g, Mean b values of Tender, Immature and Mature leaves are calculated.

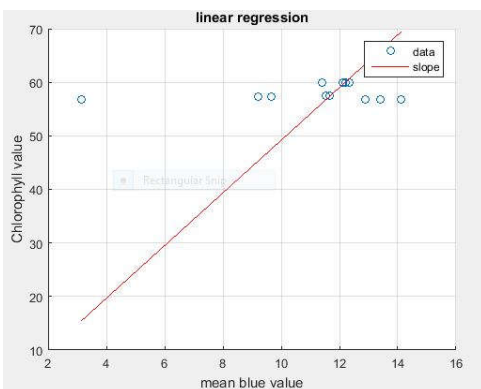
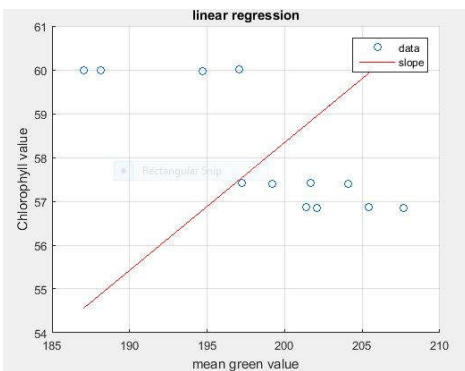
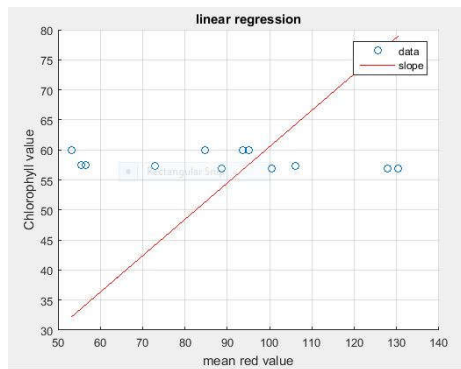
Step 4:- Mean of Total Chlorophyll Values are calculated.

Step 5:- Linear Graph for the above Calculated values found in step3 & step4 are plotted and Corresponding correlation Coefficient r values are predicted.

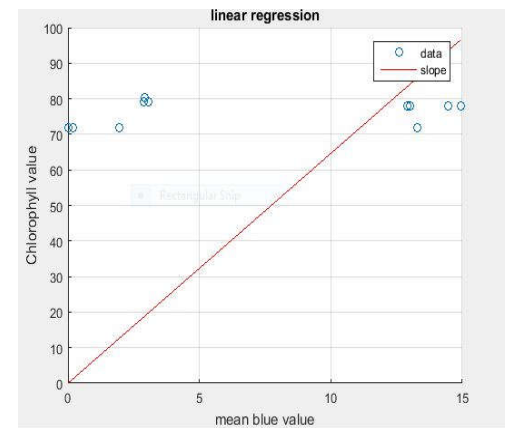
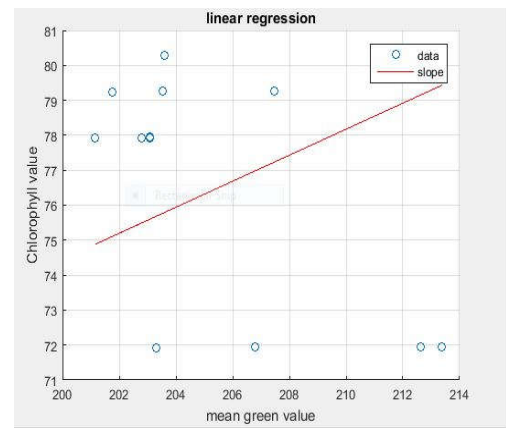
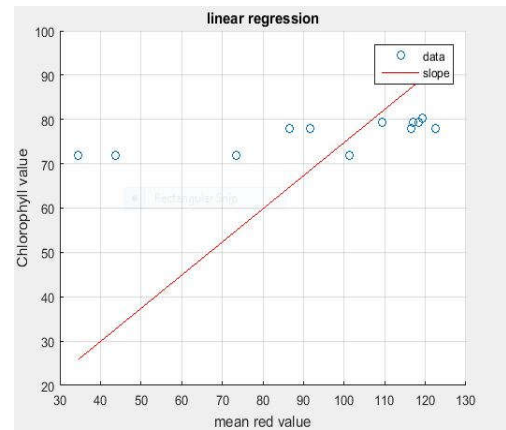
RESULT AND DISCUSSION

Greens are important sources of protective food which are highly beneficial for the maintenance of good health and prevention of diseases. In this study commonly available plant leaves were used to estimate the chlorophyll content. The Mean values of RGB values are calculated and correlated with Total Chl. values. The correlation coefficient r in tender leaves was found to be best in green with $r=-0.8444$. Similarly in Immature and Mature leaves best correlation coefficient r was found in Red with $r=-0.5331$ and $r=0.7864$ respectively.

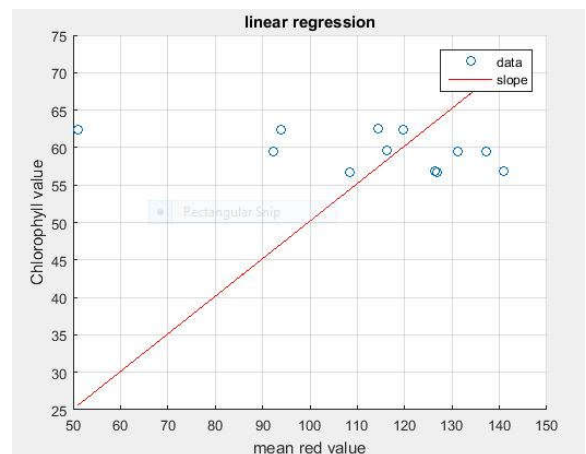
Tender Leaf

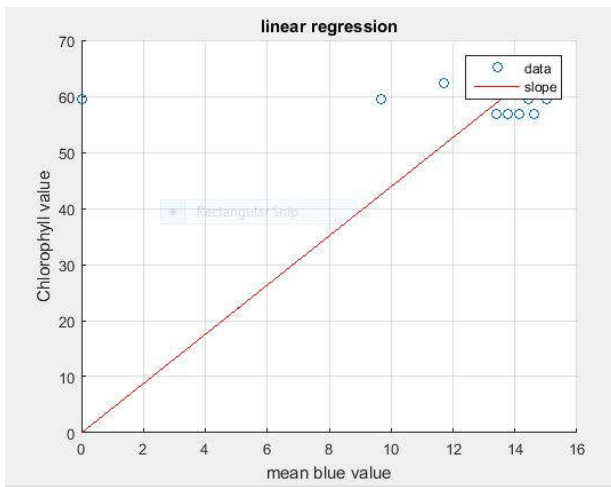
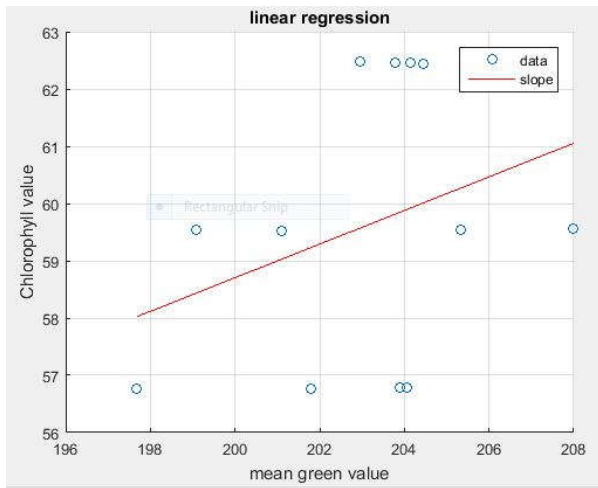


Mature leaf



Immature Leaf





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CONCLUSION

The plant material was collected from different plantation of Assam Lemon (*Citrus Limon L. Burmf*) during period of shiny morning. Before the chlorophyll estimation the midrib and hard veins are totally separated from each leaf.

In this Chlorophyll Estimation Arnon (1949) method is used for the Chlorophyll estimation. The result shows a significant variation and the correlation coefficient r in tender leaves was found to be best in green while in Immature and Mature leaves best correlation coefficient r was found in Red.

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