1   **Development of non-destructive methods to estimate functional traits and**

2   **field evaluation in tea plantations using a smartphone**

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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.

Introduction

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

Chlorophylls play an important role in the plant as primary photosynthetic pigment composed together with carotenoids exhibits a unique colour appearance which is specific to genotype and even used as a parameter of physiological maturity, nutrient status and quality (Pagola et al. 2009). The chlorophyll content is strongly related to the colour and flavour of the tea is principally determined by crop shoot chemical constituents (Wang et al., 2004) and is positively correlated with the scores for appearance and infused leaf (Wang et al., 2014). Leaf chlorophyll is mostly used as an index to diagnose diseases and nitrogen (N) status in the plants besides minor nutrient symptoms. N is the most required mineral nutrient in tea where the optimum and timely application is crucial in achieving a higher yield. However, an excess application of N in the agricultural environment can lead to water pollution and upon intake, it is transformed into nitrosamines molecules acting as 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 $j = 1, 2, \ldots, K$ by the following equations

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

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

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

109 between SPAD meter reading and image algorithm reading at each stage of measurement.

110 The coefficients of determination (R^2) indicated that there was a minor variation in

111 chlorophyll content which was explained by image processing algorithm reading at each

112 measurement stage of development (Fig.-3b) that ranging from 0.8 to 2% (Table-1). The

113 relationship between image feature and SPAD values indicated that red (R^2 = 0.830) and

114 green (R^2 = 0.857) colours are highly related with the SPAD values except for blue colour (R^2

115 = 0.006). The result also indicated the SPAD values are negatively correlated with the red (y

116 = -3.650x + 302.054) and green (y = -2.435x + 278.679) while blue is positively related (y =

117 0.107x + 13.194; Fig.-3a). The results show that the image processing algorithm readings can

118 be used as an effective indicator for evaluating the chlorophyll content of the leaf at a

119 different stage of the crop. Based on this exercise, the reader must conclude that surface

120 chlorophyll measurements are worthwhile by making use of an image processing algorithm.

121 **Measuring the nitrogen status and its relationship with yield**

122 A regression analysis was made between SPAD readings and leaf N content besides the

123 yield. There was a significant positive linear relationship between both the attributes as

124 displayed in Fig.-4. Regression analysis of the SPAD readings and yield demonstrated that

125 these attributes produced a significant linear relationship R^2 = 0.7309; y = 85.213x - 3980.2

126 (Fig.-4a). Similarly, the regression analysis of the SPAD readings and N demonstrated a

127 significant linear relationship R^2 = 0.7103; y = 0.1868x - 7.8051 (Fig.-4b). Similar results were

128 arrived by Yang et al. (2014), Ramesh et al. (2002) and Swain & Sandip (2010) who

129 established the linear relationships between leaf N content and SPAD readings and highly

130 significant correlations of SPAD readings with the yield in various crops.

131 **Clonal Characterisation**

132 The clonal signature of the UPASI released clones and an important estate selection was

133 created to identifying the superior materials from the seedling blocks or infillings of an area

134 of interest. The signature of the respective clones was established using a supervised image

135 classification method and the algorithm was applied to the images of seedling fields. The

136 plotrix of the UPASI released clones was depicted in the Fig.-5. From Fig.-6, it was observed

137 that using supervised image segmentation algorithm can be exploited for the identification

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138   and composition of infilling clones in the area or some of the elite clones like materials from

139   the seedlings block for crop improvement.

Disease assessment by Image segmentation

141   For the assessment of blister blight disease, the images were subjected to K-mean

142   hierarchical clustering and the area of infected portions was measured from the targeted

143   cluster. Fig. 7 shows the original images followed by output segmented clusters revealed

144   that the captured image can be classified into a different class of diseases. The classification

145   of images with the Minimum Distance Criterion shows that there was about 95% accuracy in

146   measuring the area of the target and the remaining 5% error consider as misclassification.

147   Form Fig.-8, it was understood that inbuilt GPS of a smartphone can be utilised for plotting

148   the disease occurrence with geographic information. This method can be used for

149   monitoring disease dynamics before and after the application fungicides on a real-time

150   basis.

Conclusion

152   The study demonstrated various non-invasive image processing methods for the

153   quantification of chlorophyll, nitrogen and disease. The methods can enable common

154   agrarian for real-time estimation and assessment of nutrients and disease and its spatial

155   dynamics for larger area management. Further study is required for validation and

156   developing an Android application for planter's utility.

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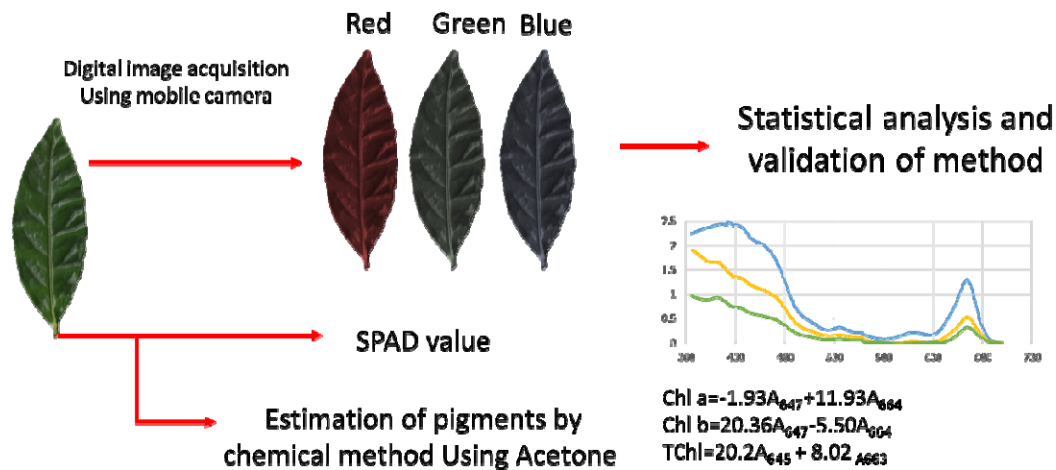182   the distribution pattern of leaf SPAD readings in the rice canopy. PLoS ONE;9: e88421.

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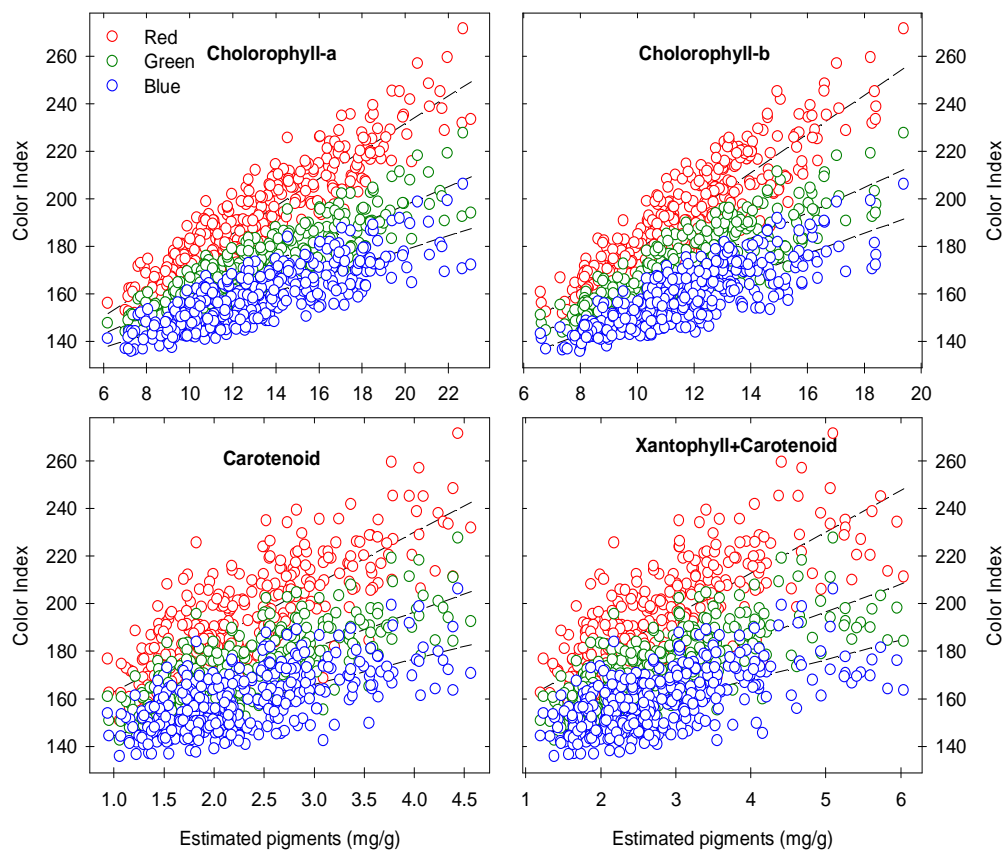184

Fig.-1: Schematic overview of the proposed study

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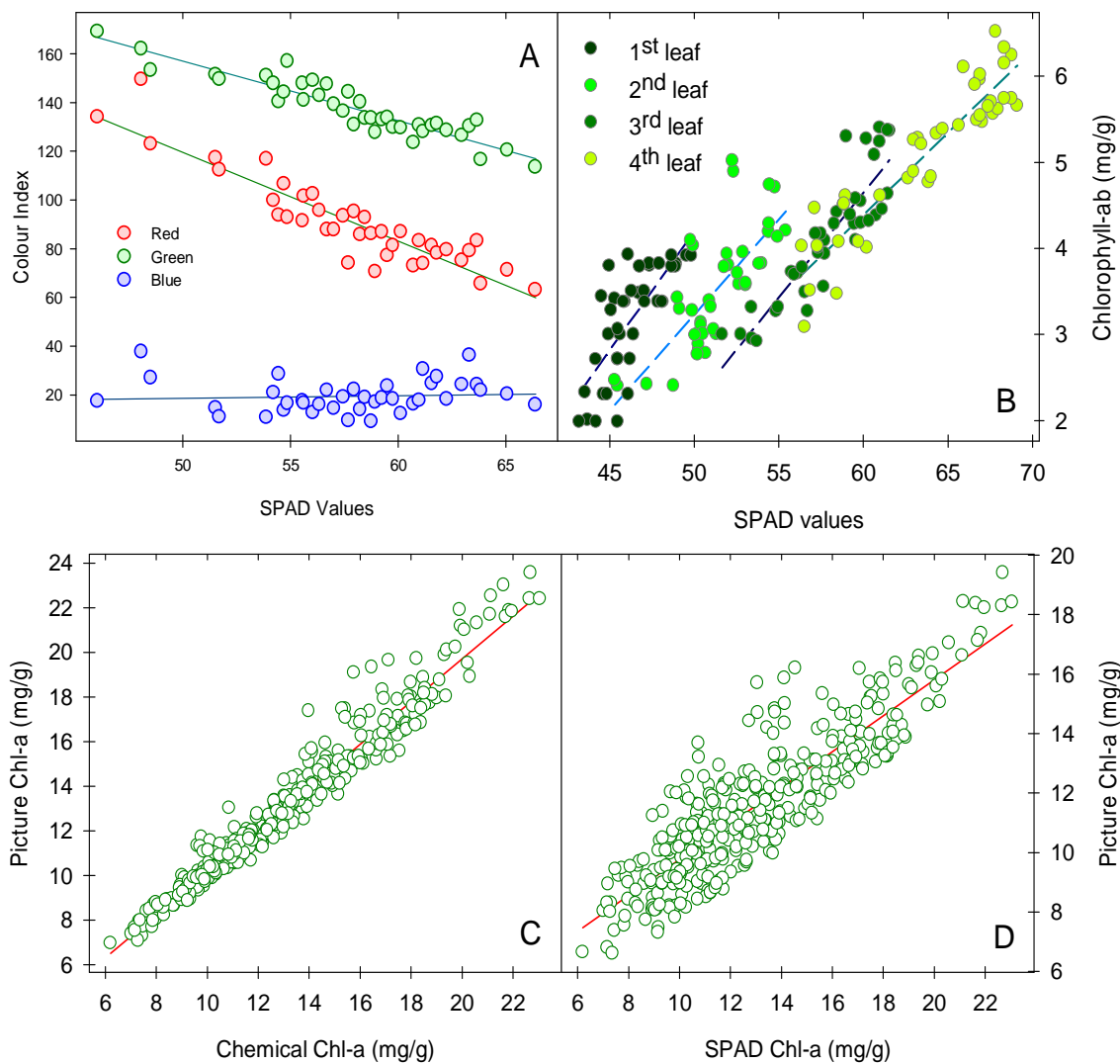
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pigments

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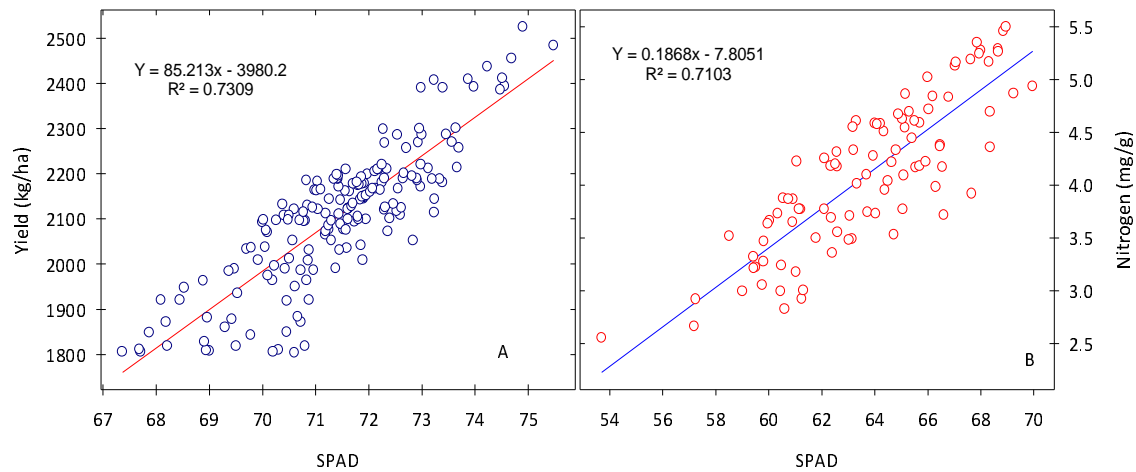
Fig.-4: Relationship between SPAD values and yield (A); and SPAD with Nitrogen (B)

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

204   Unprocessed image   Processed image
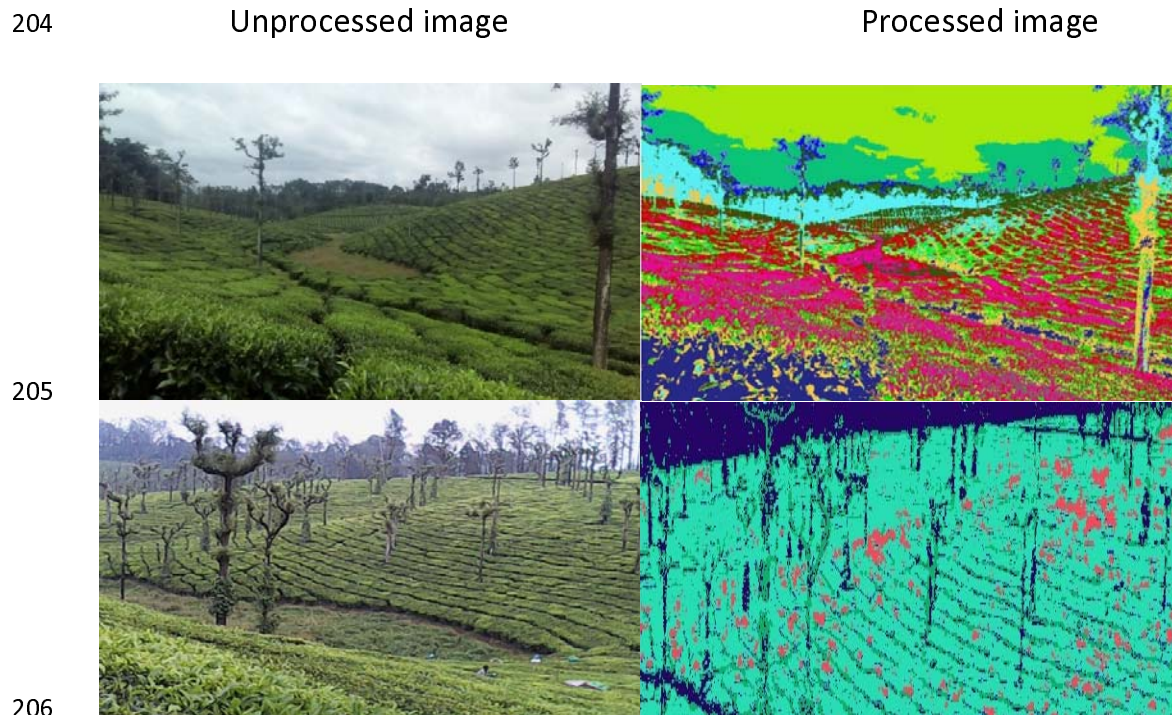
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207   **Fig.-6:** Application of supervised classification method for clonal identification

208   (top row) and elite clones like materials in the seedling block (bottom row)

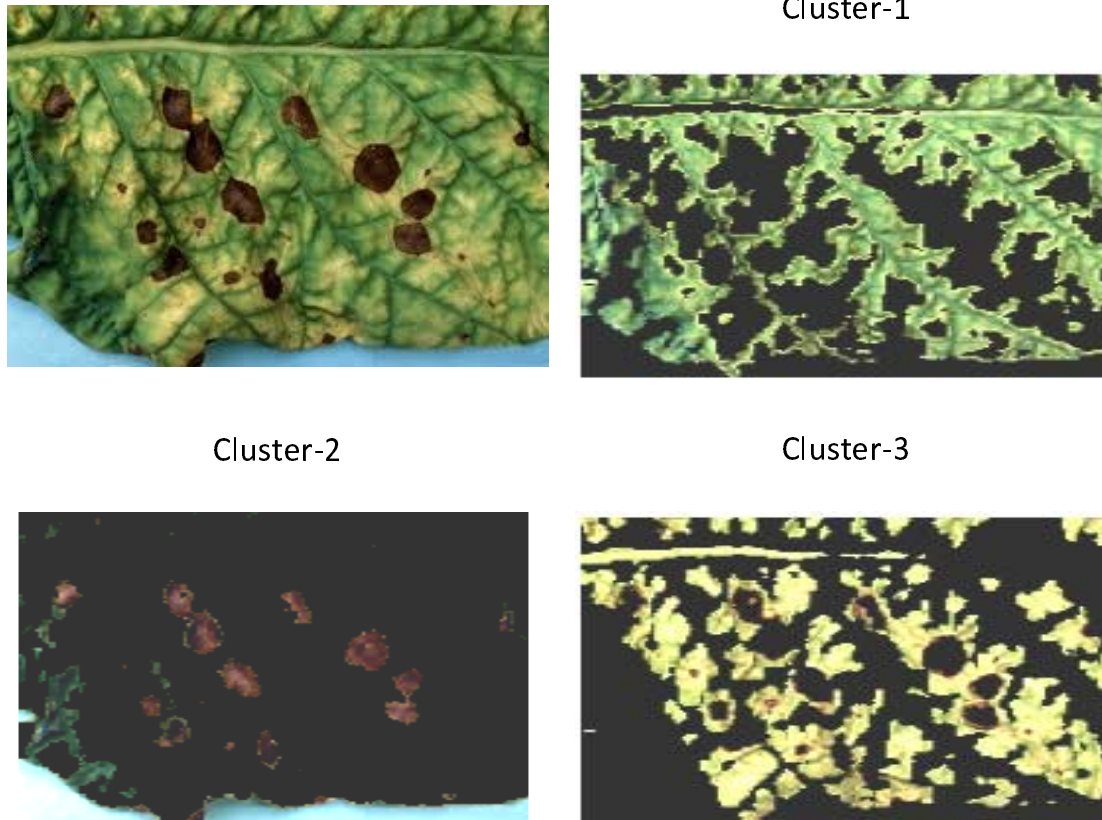209   using image segmentation analysis.

Cluster-1

Cluster-2   Cluster-3

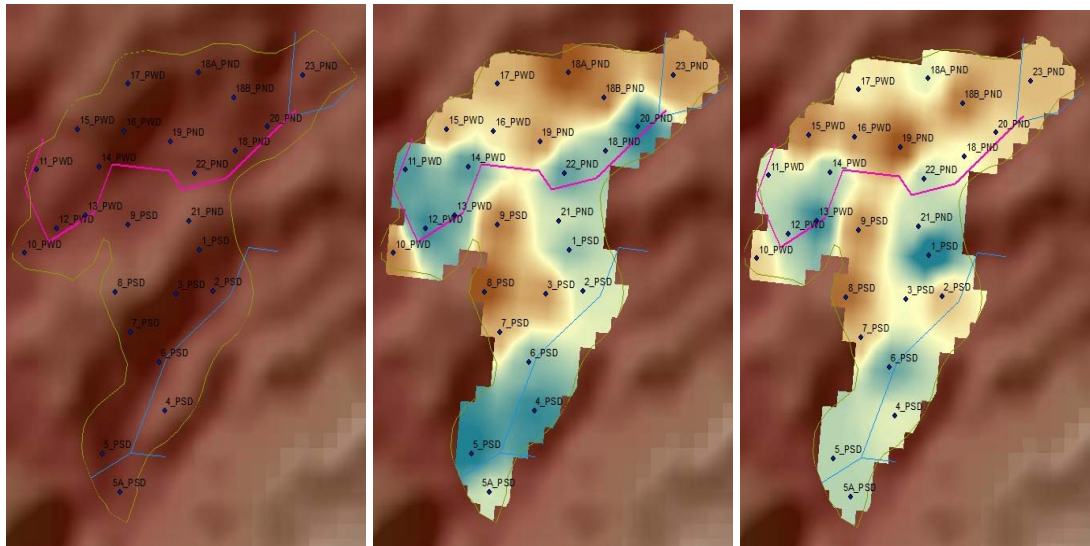**Fig.-7:** Application of image processing methods for disease assessment

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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; R^2: Correlation coefficient values; SE: standard error; and RMSE: Root mean sum square error.