

The available knowledge has shown that the sulphated polysaccharide is a wonder molecule with immense medicinal properties and it works on different types of bacteria and enveloped viruses which are similar to the SARS-CoV-2. Therefore in a world where more than 5 million infected people are struggling to get some relief and medical professionals and researchers are still fighting to reach any conclusive solution or effective medicine for SARS-CoV-2, sulphated polysaccharide from seaweeds can be a potent molecule to fight against COVID-19 pandemic. Even molecules like carbohydrate binding proteins, lectins could also be used as tool against SARS-CoV-2. A carbohydrate binding protein, Grifithsin, derived from red algae *Griffithsia* sp. – has shown *in vitro* and *in vivo* antiviral activity against enveloped viruses, and has registered low host toxicity<sup>16</sup>, making it a candidate molecule to be studied against SARS-CoV-2.

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SCIENTIFIC CORRESPONDENCE

## Estimation of leaf chlorophyll content in wheat using hyperspectral vegetation indices

Chlorophyll, the green-coloured pigment, converts light energy to stored chemical energy in the presence of water and carbon dioxide. Hence, crop yield is directly related to chlorophyll content, but large-scale determination of this parameter by conventional methods involves investment of time, money and manpower. In contrast, remote sensing, based on measuring the reflected radiation from plant canopies, plays a unique and essential role to assess chlorophyll content at different crop-growth stages in a reliable and operational way. Absorbance by carotenoids greatly restricts the use of blue peak in chlorophyll estimation. Similarly, in the red region (660–680 nm), absorption saturates at low chlorophyll content and hence is not suitable for high chlorophyll estimation. However, reflectance in the 550 and 700 nm wavelengths gets saturated at higher chlorophyll content and can be successfully used for chlorophyll estimation.

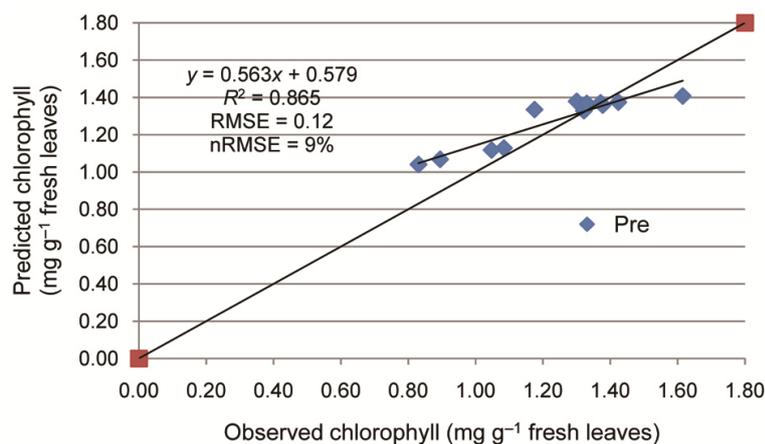
Spectral indices based on these spectral bands have been developed and successfully used for chlorophyll estimation. In the present study, we estimate chlorophyll content in wheat leaf using spectral reflectance indices in a field experiment with treatments of crop residue mulch, irrigation and nitrogen.

The study was conducted to evaluate the effect of irrigation, nitrogen and mulch in wheat at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi (77°89'N, 28°37'E, 228.7 amsl) during *rabi* season of 2012–2013 and 2013–2014. Two levels of irrigation ( $I_2$ : crown root initiation and flowering stage;  $I_4$ : crown root initiation, tillering, flowering and grain filling stage) were imposed as main plot along with two levels of mulch ( $M_0$ : without mulch,  $M_1$ : with maize straw mulch @5 t ha<sup>-1</sup>) as sub-plot and three levels of nitrogen ( $N_0$ : no nitrogen,  $N_{60}$ : 60 kg N ha<sup>-1</sup>,  $N_{120}$ : 120 kg N ha<sup>-1</sup>) as sub-sub-plot. The

total nitrogen amount was applied in three growth stages (sowing, 50%; CRI, 25% and flowering, 25%), whereas total P and K were applied at sowing during the cropping season. All the treatments were laid out in split-split plot design.

The spectroradiometer (ASD FieldSpec, Analytical Spectral Devices Inc., USA) was used to measure reflectance (350–2500 nm) of plant canopy during 11.00 to 13.00 h on sunny days. All the measurements were taken at 1 m distance from the top of the plant with 25° field of view. The spectral signature and leaf chlorophyll content of the crop were observed at flowering stage. The reason for choosing flowering stage is that leaf chlorophyll content is highest at this stage and then decreases towards grain-filling stages due to dilution effect. The leaf chlorophyll content was measured by DMSO method.

The performance of the model was assessed by comparing the statistics ( $R^2$ ,



**Figure 1.** Observed and predicted chlorophyll content using the RGRcan model.

coefficient of determination; RMSE, root mean square error and nRMSE, normalized root mean square error). A higher value of  $R^2$  indicates closeness of the observed data to the line of best fit, whereas lower RMSE and nRMSE indicate better model performance. The model performance was considered excellent, good, fair and poor for nRMSE values of <10%, 10–20%, 20–30% and >30% respectively.

The reflectance of wheat under different N treatments ( $N_0$ ,  $N_{60}$  and  $N_{120}$ ) showed marked differences throughout the spectral region (350–2500 nm) of measurement. The reflectance in the visible range (400–700 nm) was highest in  $N_0$  followed by  $N_{60}$  and  $N_{120}$ . Reduction in nitrogen dose reduces leaf chlorophyll content, which increases reflectance in the visible region due to decreased radiation absorbance. However, in the NIR region (700–1300 nm), the highest canopy reflectance was observed in  $N_{120}$  followed by  $N_{60}$  and  $N_0$ . This might be due to the higher LAI in  $N_{120}$  compared to  $N_{60}$  and  $N_0$ . In the SWIR region (1300–2500 nm), higher reflectance was observed in  $N_0$  than  $N_{60}$  and  $N_{120}$ , probably due to lower water content of plant canopy in  $N_0$ . The reflectance values of  $N_{60}$  and  $N_{120}$  treatments were almost similar. The spectral reflectance values under visible, NIR and SWIR in irrigation treatments ( $I_2$  and  $I_4$ ) and mulch treatments ( $M_0$  and  $M_1$ ) were statistically at par. This could be attributed to similar chlorophyll, LAI and leaf water content under irrigation and mulch treatments.

The irrigation and mulch treatments did not affect chlorophyll content signi-

ficantly during the study years. However, nitrogen treatments significantly ( $P < 0.05$ ) influenced leaf chlorophyll content. During 2012–2013, the  $N_{120}$  (1.391 mg g<sup>-1</sup> fresh leaves) treatment registered highest leaf chlorophyll content followed by  $N_{60}$  (1.138 mg g<sup>-1</sup> fresh leaves) and  $N_0$  (0.990 mg g<sup>-1</sup> fresh leaves) treatments. In 2013–2014, the  $N_{120}$  (1.429 mg g<sup>-1</sup> fresh leaves) treatment registered significantly higher leaf chlorophyll content compared to  $N_0$  (0.964 mg g<sup>-1</sup> fresh leaves) treatment. However, chlorophyll contents in  $N_{120}$  and  $N_{60}$  (1.300 mg g<sup>-1</sup> fresh leaves) were statistically similar. Nitrogen is an essential constituent of chlorophyll molecule and part of the enzyme associated with chlorophyll synthesis. Hence, application of N increases leaf chlorophyll content.

Nineteen spectral reflectance indices (SIPI, Ratcart, PRI, PSRI, MCARI, TCARI, SR705, ND705, mND705, mSR705, Readone, RGRcan, NDVI-canste, MTCI, DD, Green model, Red edge model, GI and TCI) commonly used for chlorophyll estimation were collected from the literature. Predictive equations were developed for assessment of chlorophyll status over crop canopies using selected spectral indices. The best-fit equation (exponential or logarithmic or power regression or linear) with highest correlation coefficient ( $r$ ) and coefficient of determination ( $R^2$ ) was selected as the best predictive equation. Out of the 19 spectral indices, three (MCARI, TCARI and TCI) were not significantly correlated with leaf chlorophyll content. The spectral reflectance indices SIPI, Ratcart and PSRI were significantly ( $P \leq 0.01$ )

negatively correlated with leaf chlorophyll content, and Ratcart had highest correlation coefficient of  $-0.765^{**}$ . The remaining 13 were significantly ( $P \leq 0.01$ ) positively correlated, of which MTCI had the highest correlation coefficient ( $r$ ) of  $+0.893^{**}$ . These regression models (predictive equations) were evaluated based on the datasets obtained during 2013–2014. The coefficient of determination ( $R^2$ ) varied from 0.011 (SIPI) to 0.877 (PRI), RMSE from 0.12 (RGRcan and GI) to 1.25 (Green model), and nRMSE from 9% (RGRcan) to 102% (Green model). The regression model having the highest  $R^2$  and lowest RMSE and nRMSE values was considered as the best model to predict leaf chlorophyll content. The spectral reflectance indices MCARI, TCARI and TCI were not evaluated as their predictive equations were not statistically significant. Considering  $R^2$ , RMSE and nRMSE values, it is recommended that the regression model based on spectral reflectance index RGRcan can be successfully used for estimation of leaf chlorophyll content (Figure 1). This model could account for 86.5% variation in leaf chlorophyll content with minimum RMSE (0.12 mg g<sup>-1</sup> fresh leaves), which is 9% of the mean observed chlorophyll content.

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