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Assessment of Chlorophyll Content in Leaves of Crops and Orchards Based on SPAD, Multispectral, and Hyperspectral Techniques

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Abstract. Strategic planning in developed and developing countries has significantly benefited from early assessment, identification and crop production monitoring. Remote sensing surveillance of crop health has brought significant benefits to farmers regarding early detection of latent issues, such as nutrient deficiencies or crop ailments, and taking remedial action. The study used geospatial techniques to monitor the orchards and crops of Halani in the Pakistani province of Sindh, using GeoEye and Landsat-8 satellite imagery. The absorbance of chlorophyll content in six fruit trees: mango (*Mangifera indica* L.), banana (*Musa acuminata* Colla), musambi (*Citrus limetta* Risso), kino (*Citrus aurantium* L.), lemon (*Citrus limon* (L.) Osbeck) and guava (*Psidium guajava* L.), as well as four crops: maise (*Zea mays* L.), rice (*Oryza sativa* L.), cotton (*Gossypium herbaceum* L.*)*, and sugarcane (*Saccharum*) were recorded spectrophotometrically using a Beckman Coulter DU-530 single cell module spectrophotometer at 648 nm and 665 nm (homogenised in 100% ethanol), and non-destructive chlorophyll using a SPAD-502 portable chlorophyll meter (Minolta Corporation, New Jersey, USA) showed a strong positive correlation**.** The results of chlorophyll absorbance showed the same trend in crops through satellite data and laboratory analysis. Chlorophyll content and NDVI showed a positive correlation. The R^2 value for rice and banana was 0.9925 and 0.9578, respectively, while the SPAD and chlorophyll R^2 for rice was 0.838 and 0.75 for banana. The overall results indicate high chlorophyll content in the leaves of orchards rather than crops. The study's outcomes show that satellite data are a potentially reliable and resourceful tool for early assessment of the reliability of agricultural monitoring. The health and growth of crops can be monitored with satellite data, which are ultimately used for yield prediction, consequently helping growers strategically harvest and market.

Keywords: crops, fruit trees, NDVI, Landsat-8, spectral profile, chlorophyll, orchards.

1. Introduction

Primary valuation and precise monitoring of crops condition and production significantly deliberately assist the planning for the established and emergent nations (Sinha & Dhanalakshmi, 2022; Wu et al., 2023). Worldwide increase in temperature, erratic patterns of rainfall, surging of flood levels, and drought critically affect the agriculture sector than other industries (Chandio et al., 2020; Ahsan et al., 2020; Ishaque et al., 2022). Climate change and urban sprawl creating chaotic condition and peering pressure intensify the demands of cereal crops (Chandio et al., 2023). Abubakar (2020) noted, increasing temperature shifted monsoon pattern in Pakistan which ultimately amplified manifestation of cyclones, effects critically to the agricultural sector. According to Ahmad et al. (2015) in vulnerable index, Pakistan ranks fifth most susceptible country, which is greatly affected by climate change. Over burden of population increases the demand for urban land creating devastating consequences in Pakistan (Anwar et al., 2020; Munawar et al., 2023). As economy of Pakistan depends significantly on agriculture chiefly cereal crops, it's a dire need to valuate crop yield as early as possible. Hassan and Goheer (2021), applied multispectral vegetation indices using remote sensing and GIS techniques and predicted precise crop yield two months prior to harvesting. Gumma et al. (2020) calculated the aptitudes and parameters of mapping in the cultivated areas in the Rabi season conforming patterns of crops.

In order to identify various crop varieties Remote sensing data plays a crucial role. In their recent study, Snevajs et al. (2022) anticipated a method aimed at enabling the supervised categorization of Sentinel-1 and Sentinel-2 data for the purpose of crop type identification. Remote sensing technology enables the acquisition of vast quantities of data within a condensed timeframe. Remote sensing techniques has distinct characteristics that enable the acquisition of geographical and temporal data pertaining to various regions and events in a comprehensive manner. The increasing need for innovative, scientific, and technical assessments has proven beneficial for cultivators in improving plant health, agricultural sustainability, and overall efficiency. Remote sensing technologies offer new possibilities for scientists to integrate biology with smart agriculture in order to achieve increased crop yields and reduced inputs in plantations (Ennouri & Kallel, 2019).

Various satellite crop detection methods can be opted to identify diverse categories of field crops, thereby contributing to the development of food security. Remote Sensing technologies had been extensively used by various global organizations, GEO monitoring, (FAO, Food and Agricultural Organization) agriculture production monitoring, and the common agriculture policy (Whitcraft et al., 2015; Schmedtmann & Campagnolo, 2015; Reynolds et al., 2000), along with local sectors such as business owners, food industries, for strategic policymaker, sustainability forecasting, and investment (Rembold et al., 2013). Multispectral satellite images are used for the recognition of crops ultimately develop Thematic maps by processing data through image classification, pixel based supervised classification theoretically more accurate (Jog & Dixit, 2016; Boori et al., 2018; Pech-May et al., 2022).

In the agricultural sector since several decades, soil indices, water indices and vegetation indices derived from multispectral sensors have been extensively utilized. These indices play a crucial role in monitoring crop health and aiding farmers in making informed decisions. Spectral indices are derivative of mathematical combination of two or more spectral bands, resulting in a merged single value (Gu et al., 2011). Matsushita et al. (2007) proclaim that Normalized Difference Vegetation Index (NDVI) is commonly used indices. The Normalized Difference Vegetation Index (NDVI) has been found to have a positive correlation with many physiological parameters of plants, including photosynthetic efficiency, potassium content, phosphorous, foliar nitrogen, and leaf chlorophyll content (Shrestha et al., 2023). Additionally, NDVI has been associated with the overall greenness of vegetation (Xue et al., 2023).

Spectroscopy is a very accurate and non-invasive methodology that provides valuable insights into many physiological processes occurring within plants and trees. Once incorporated into compact portable devices, it has the capability to deliver immediate and precise outcomes inside various settings such as field environments, forests, and laboratory settings. Currently, there is a limited amount of research conducted on the subject region utilizing GIS and spectrometer methodologies. The purpose of the study was to determine the agricultural fields in the designated study area based on the predominant crops present. Additionally, the study aimed to compare the chlorophyll content in various crops and orchards using satellite imagery analysis. Furthermore, the study sought to create a normalized difference vegetation index (NDVI) and examine its spatial distribution. Finally, the study aimed to establish a correlation between in-situ data and satellite data in order to identify the relationship between NDVI and chlorophyll values.

2. Materials and methods

2.1. Research field

The study region under consideration is Peer Wario, Halani, of District Naushahro Feroze in the province of Sindh, Pakistan. It is geographically situated at coordinates

27°05′ 11” N and 68°18′ 47” E, as seen in Figure 1. The accurate forecasting of weather conditions in a given region significantly influences agricultural productivity. The climatic conditions in District are conducive to the cultivation of various crops including as maize, wheat, rice, and orchard fruits including mango, orange, and guava, among others. According to Brewer et al. (2022), the summer season experiences temperatures reaching as high as 45°C, while the winter season sees temperatures dropping as low as 5°C. The annual precipitation levels are consistently low, with an average rainfall of 45 mm during the summer season.

The region exhibits a combination of mechanized agricultural practices alongside the utilization of traditional ways. The farmers employed a weekly irrigation method called „Wara Bandi" to hydrate their fields using canal water. The implementation of an unlined irrigation system resulted in a detrimental scenario for the farmed land, leading to the transformation of the area into a saline environment. Farmers are adapting the issue of waterlogged areas by transforming them into fish farms. Therefore, these regions are exhibiting a notable attraction for avian species native to Siberia. Salinity is a global concern, resulting in the transformation of vast expanses of land into barren terrain.

2.2. Data collection

A GPS-coordinated survey was conducted to collect samples of leaves from the fields containing maize (*Zea mays* L.), kino (*Citrus aurantium* L.), cotton (*Gossypium herbaceum* L.), mango (*Mangifera indica* L.), rice (*Oryza sativa* L.), lemon (*Citrus limon* (L.) Osbeck), musambi (*Citrus limetta* Risso), sugarcane (*Saccharum*), banana (*Musa acuminata* Colla) and guava (*Psidium guajava* L.). The botanical nomenclature of the studied plants is given according to World Flora Online (WFO, 2023).

Satellite data of Landsat-8 was attained in 2014 from the USGS-Earth Explorer. The cartographic representation of the study area has been obtained from the Pakistan Bureau of Statistics. Scanned image has been georeferenced and digitized through ArcGIS 10.3.1.

2.3. Quantification of chlorophyll using the SPAD method

Portable Chlorophyll meter SPAD-502 (obtained data in arbitrary units) was used to estimate chlorophyll content. The quantification of chlorophyll content in wheat and orchard leaf samples was conducted using a conventional methodology.

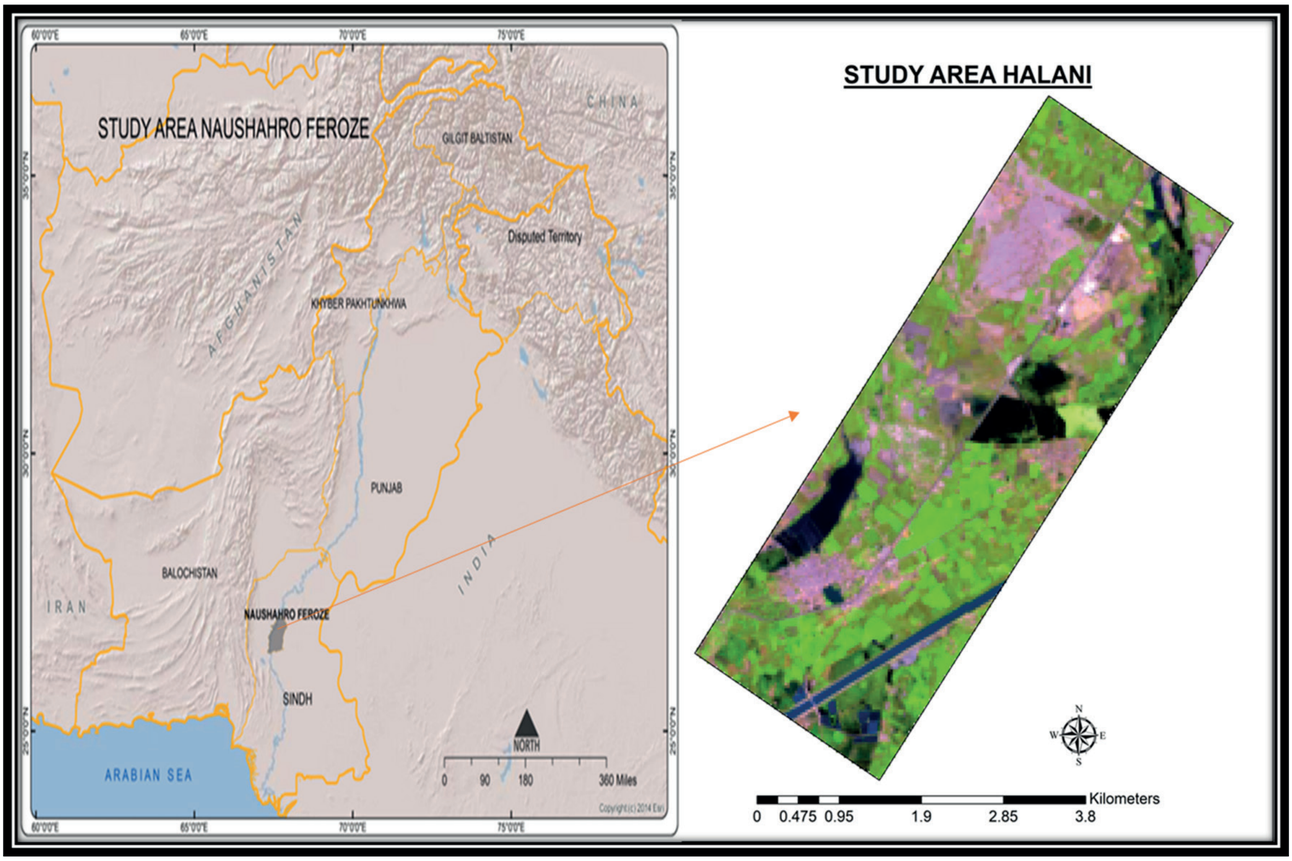

Figure 1. Study area

2.4. Quantification of chlorophyll content using a spectrophotometer

The leaf chlorophyll concentration of various plant species, was determined through extraction from a 95% ethanol solution and subsequent spectrophotometric analysis. The Beckman Coulter DU-530 single cell module Spectrophotometer was employed for this purpose, following the methodology outlined by Ritchie (2006). The leaves were carefully weighed and thereafter placed into 2 ml Eppendorf tubes that were pre-filled with a solution of 95% ethanol. Please ensure that all Eppendorf tubes are thoroughly mixed using a Vortex meter 300. To mitigate evaporation, it is advisable to employ aluminum foil to cover the tubes. Subject the individual to a period of sensory deprivation lasting for a duration of three consecutive days. The chlorophyll extract was poured into a test tube and left undisturbed for a duration of three days, resulting in the collection of a total volume of 3 milliliters. Measuring of optical density through spectrophotometer at wavelengths of 664.2 nm and 648.6 nm, as described by Gitelson et al. (2003). The determination of chlorophyll content in leaves is precisely assessed by the method, an extraction procedure with an organic solvent ethanol (Sumanta et al., 2014). The obtained values are then translated to chlorophyll content utilizing Equation 1.

The equation (1) can be expressed as

$$Chl(a+b) = 5.24A664.2 + 22.24A648.6,$$

where A represents the absorbance.

2.5. The normalized difference vegetation index (NDVI)

The NDVI is extensively used remote sensing technique that computes the vegetation's existence and health. This vegetative Index, Al-lami et al. (2021), efficiently discriminates green vegetation from the adjacent soil background (Fig. 2). The image was clipped using ArcGIS 10.3.1 software. Subsequently, through Raster Calculator tool the NDVI of Landsat 8 calculated using the, Equation 2:

$$NDVI = (Band5 - Band4) / (Band5 + Band4)$$

The Band-5 also known as Near Infrared Band (NIR) and Band-4 the Red Band are specific spectral bands of Landsat-8. NDVI values range from +1 to -1, where -1 is typically water and +1 is characteristically dense, lavish

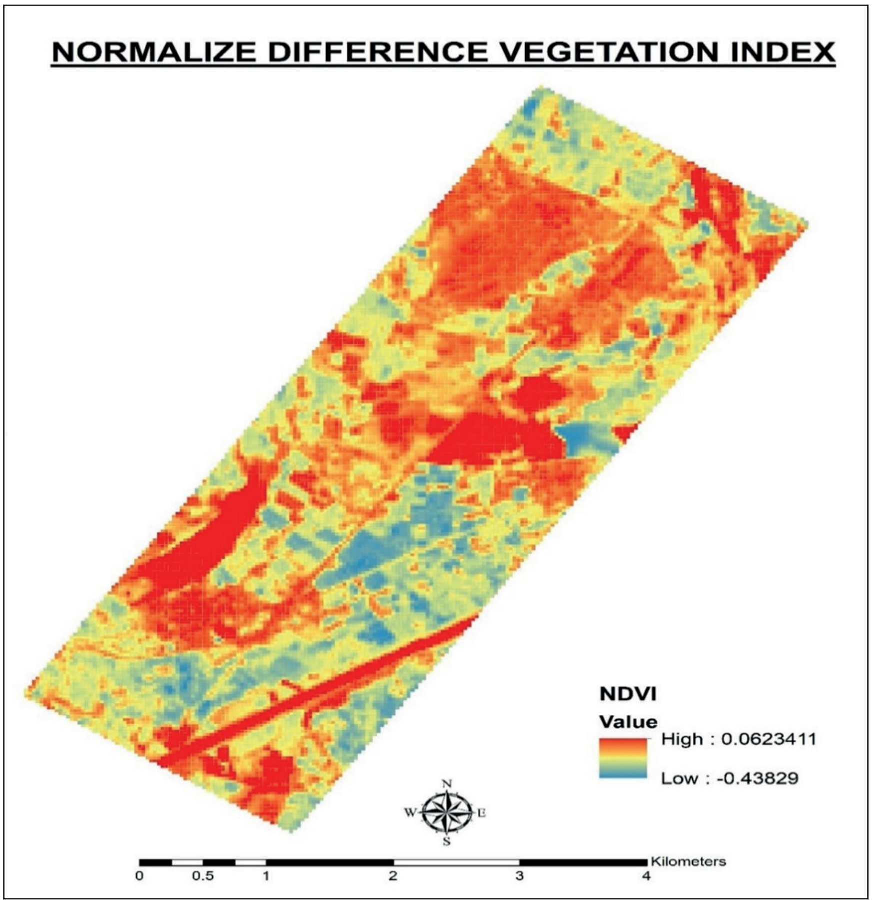

Figure 2. Normalize difference vegetation index

vegetation. Consequently, NDVI can indicate healthy vegetation. Formally, NDVI was given by Braun and Herold (2004).

2.6. Determination of chlorophyll concentration

The determination of chlorophyll concentration was performed by employing the specific absorption coefficients for chlorophyll a and b as published by Fassnacht et al. (2012). A random sampling technique was employed to obtain representative samples from each crop field, which were then geographically coordinated using GPS technology. The quantities of chlorophyll a, chlorophyll b, and carotenoids have been found to be positively associated with a plant's photosynthetic potential and can provide insights on the physiological condition of the plant (Falcioni et al., 2023; Strzałka et al., 2003). The primary factor influencing chlorophyll concentration is nitrogen availability, as indicated by (Hill et al., 2016; Wang et al., 2023). The chlorophyll content can be determined using raster data with the Raster Calculator of Map Algebra, as described in Equation 3.

The equation (3) can be expressed as $y = a + b * x$.

In this study, the variables are defined as follows: y represents the chlorophyll content on a raster cell, while a and b represent the total chlorophyll values at wavelengths 665 nm and 648 nm, respectively. Lastly, x denotes the Normalized Difference Vegetation Index (NDVI).

2.7. The image classification

The supervised classification is principal method for (LULC, Land use land cover) wherein the experts classify homogeneous pixels of the areas of interest within the image. According to Luo (2021), images transmit a lot of data and perform chief role, as it's important to get valuable picture info within real time. In this process, classification algorithm is used to regulate the classes of image. The image classification's core progression is image feature extraction, image preprocessing and classifier design. Selected samples are commonly denoted as training regions. The choice of training locations is determined by the performer's level of acquaintance with the geographical region and their understanding of the specific surface cover types depicted in the image. Therefore, the analyst is overseeing the process of categorizing distinct classes. All the sample with same pixels merged into one class as the system is trained to recognize spectrally comparable areas. In the context of supervised classification, the initial step involves the identification of information classes, which are subsequently utilized to derive the corresponding spectral classes.

2.8. Spectral Reflectance

In order to measure the leaves spectral reflectance seven samples of individuals per species, randomly chosen. Spectral reflectance of leaves measurements through Field Spec AVASPEC-2048-3-DT Avantes. The laboratory conducts spectral measurements within a spectral range spanning from 200 to 1100 nm. The acquisition of the leaf's reflectance spectra can be achieved by calculating the ratio between a reflective white standard and the spectral radiance. Rosevear et al. (2001) elaborate that the pigments present in leaves have the ability to absorb light within the wavelength range of 400-700 nm, resulting in a decrease in the reflection of (PAR, Photosynthetically Active Radiation). The absorption pattern of the pigment plays a crucial role in governing the unique reflectance signatures exhibited by leaves. At the level of the canopy, the reflectance is influenced by the reflectance of individual leaves and their texture, whereas the determination of leaf reflectance is based on the chemical composition (El-Hendawy et al., 2022). Various reflectance indices can be employed to calculate biophysical factors, including biomass, photosynthetic size, radiation use efficiency, and water content, based on reflectance spectra.

3. Results and discussion

Significant relationships were observed between the SPAD (Non-destructive) measurements and the laboratory chlorophyll content (Destructive) in the study. The R^2 values for the different crops, namely maize, guava, cotton, sugarcane, mango, lemon, kino, musambi, rice, and banana, are as follows: cotton (R^2=0.93), guava (R^2=1), maize (R^2=1), lemon (R^2=0.91), sugarcane (R=0.92), kino (R^2=0.99), mango (R^2=0.94), musambi (R^2=0.84), banana (R^2=0.75) and rice (R^2=0.84). These results are illustrated in Figure 3. Though the destructive technique is more precise as compare to the nondestructive technique, study shows no significant variation which, may associated to the genetic properties of plant (Ali et al., 2021).

In their study, Zhu et al. (2012) conducted a comparison between SPAD measurements and laboratory leaf chlorophyll values for several crop species. The findings of their investigation revealed robust relationships between laboratory leaf chlorophyll content and SPAD measurements. The utilization of remotely sensed data is a common practice in several applications such as LCLU mapping, evaluation of resources, management of land, vegetation mapping and modelling (Booth & Tueller, 2003; Hosseini et al., 2004; Henebry, 2011). The correlation among satellite images and ground-based data is contingent upon several aspects, including the time of recording, the level of precision in the imagery, as well as both biological and non-biological elements (Huang et al., 2002; Wang et al., 2006; Soudani et al., 2012).

The normalized difference vegetation index (NDVI) is widely recognized as the most commonly employed vegetation index. The utilization of Landsat satellite data is widespread in the computation of vegetation indices such as NDVI (Fig. 2). Additionally, it serves as a means to monitor the status of both cultivated and natural vegetation, as well as to identify occurrences of desertification, drought, and deforestation (Zhang et al., 2022).

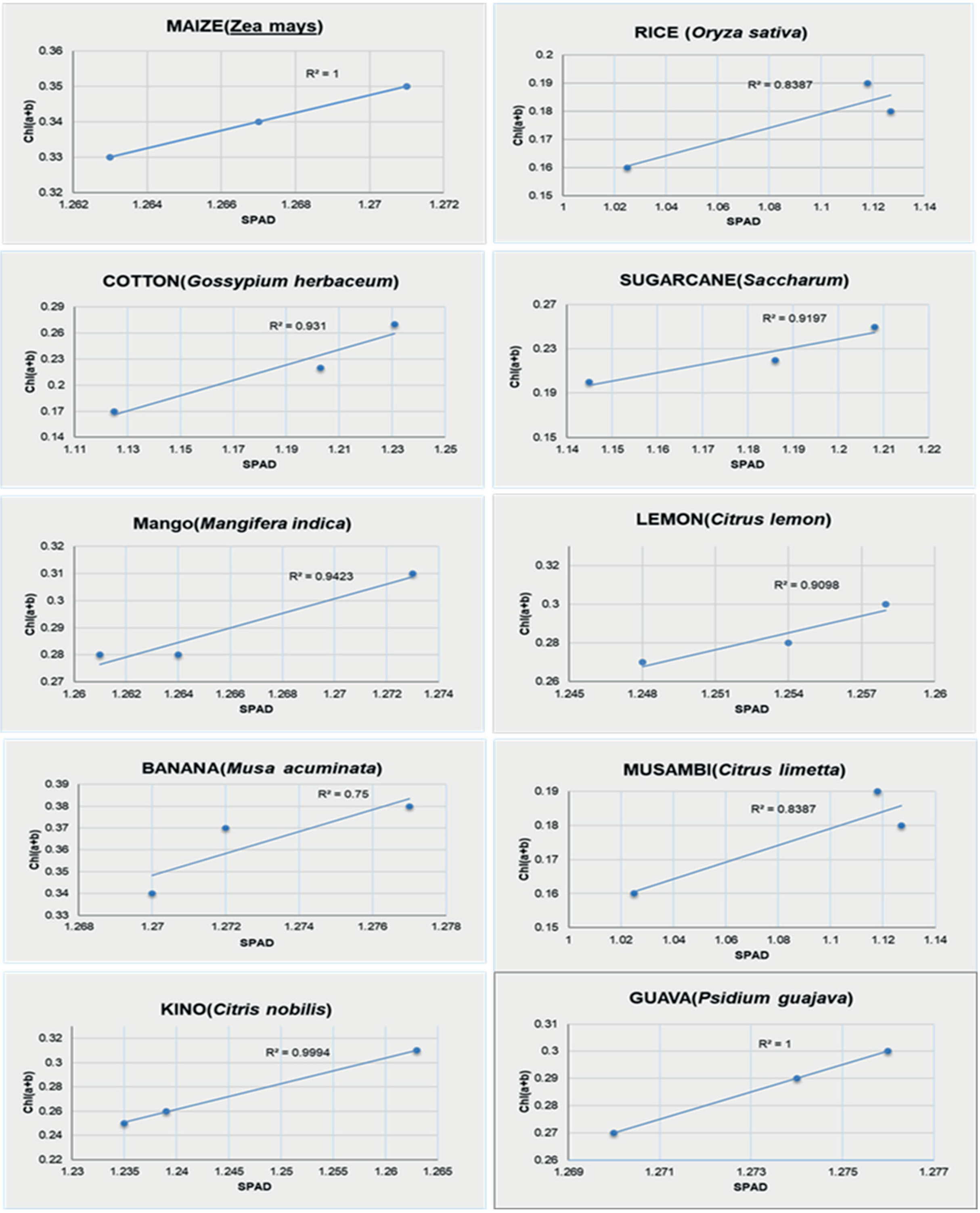

Figure 3. Relationship of total Chl(a+b) and SPAD

The coefficients of determination for NDVI and Chlorophyll revealed that normalize difference vegetation index exhibited a strong positive correlation with laboratory measurements of chlorophyll concentration in both rice (R^2=0.99) and guava (R^2=0.99). Conversely, mango (R^2=0.88), lemon (R^2=0.82) and banana (R^2=0.96), displayed significant negative correlations with NDVI respectively. In contrast, the estimation of chlorophyll concentration exhibited a weak correlation with NDVI in sugarcane (R^2=0.15), cotton (R^2=0.10), kino (R^2=0.30), musambi (R^2=0.37), and indicating a non-significant association. On the other hand, maize has no correlation whatsoever (R^2=0) as depicted in Figure 4. According to Jones et al. (2007), the multispectral imaging system demonstrated sensitivity to variations in chlorophyll and biomass output as observed through the analysis of NDVI data.

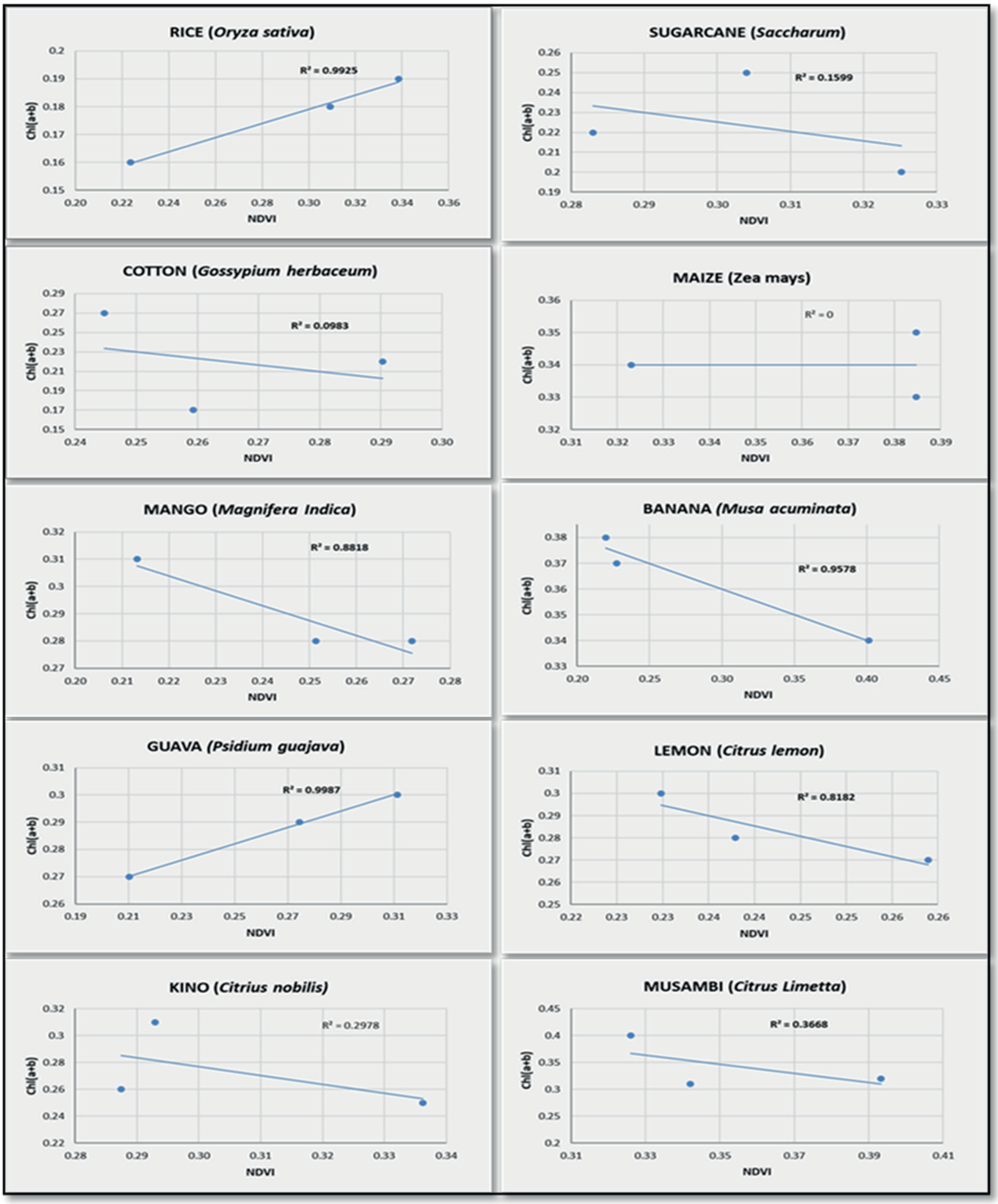

Figure 4. Relationship among NDVI and Chl (a+b)

A correlation analysis was conducted to ascertain the correlation coefficient (r) amongst the reflectance and chlorophyll content. According to Davies (2009), chlorophyll a and chlorophyll b are the foremost types of chlorophyll found in plants having properties of absorbing red and blue light. Chl a and Chl b exhibit distinct absorption points crucial for photosynthesis, occurring at wavelengths of 663 nm and 426 nm for chlorophyll a, and 645 nm and 455 nm for chlorophyll b, respectively. In the regression analysis of the chlorophyll model based on NDVI, the NDVI readings were designated as the independent variable, while the ethanol-extracted chlorophyll was designated as the dependent variable. The data was organized into a tabulated format and afterwards transformed into a raster image. The map illustrates that chlorophyll levels are significantly higher, specifically at a value of 16.11, in cultivated regions, whereas they are comparatively lower, with a value of 18.86, in barren land and water bodies (Fig. 5).

The region of interest depicted in (Fig. 6), demonstrates that the study area exhibits a high level of productivity in terms of crop and orchard production. The findings of this study demonstrate that supervised image classification has successfully recognized six distinct categories, including orchards, crops, barren terrain, salty pond, fresh water, and residential area. This identification process was facilitated using interactive image classification, which also enabled the calculation of the relevant areas for each category.

The detection of plant species by satellite remote sensing poses challenges, as it is a complex task. In order to identify plant species without causing any harm to

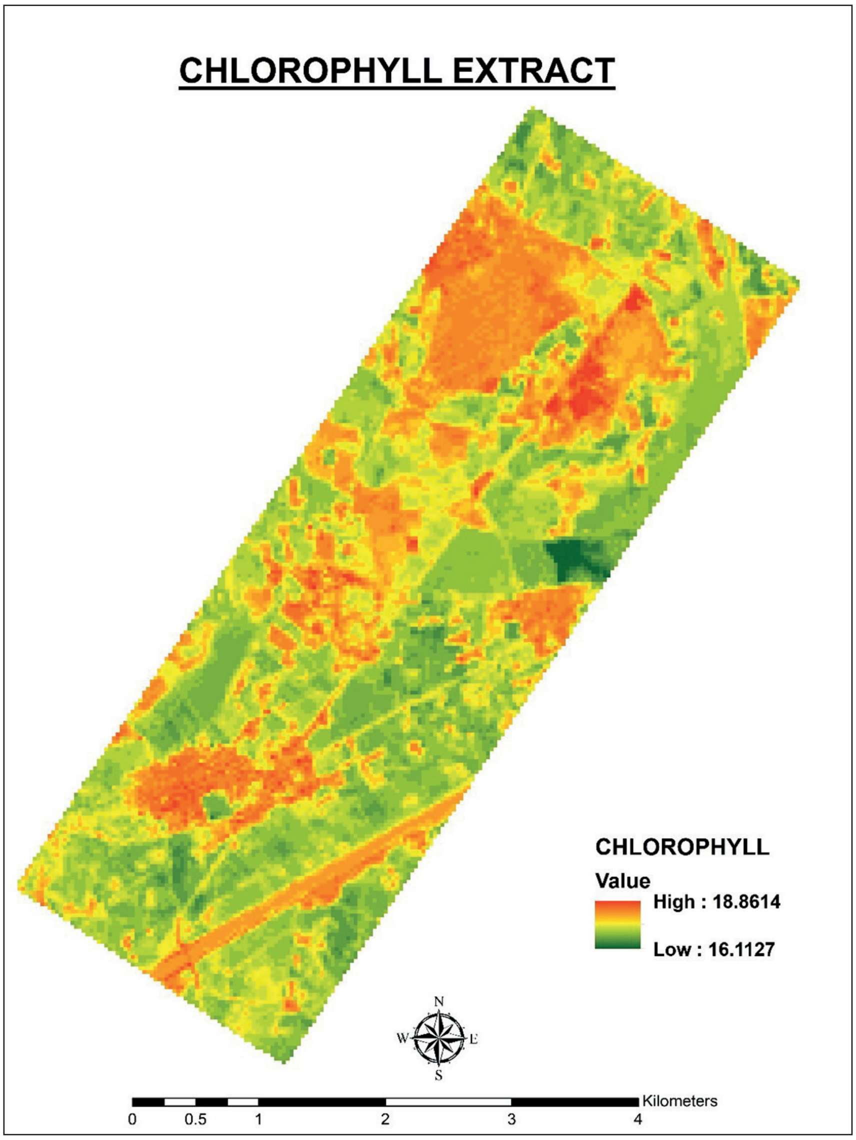

Figure 5. Chlorophyll extraction

them, it becomes imperative to develop a spectral profile for each individual species. The spectral characteristics of plant leaves exhibit a higher degree of sensitivity towards variations in chlorophyll concentration as opposed to the Normalized Difference Vegetation Index (NDVI). Indices in higher plants serve as indicators of disease, stress, and senescence. Narmilan et al. (2022) stated that ratio vegetation index (RVI), and difference vegetation index (DVI) showed a strong and positive association with the greenery content of sugarcane crops.

The data in Figure 7 displays the spectrum reflectance patterns of cotton, maize, rice, guava, mango, and banana. The regions of maximal sensitivity for chlorophyll concentration are observed at wavelengths of 550 nm and 750 nm. The spectrum reflections of a plant vary depending on factors such as plant kind, age, development stage, percentage of coverage, biomass, and water content within the cell (Coops et al., 2003; Jeganathan et al., 2010). The primary function of chlorophyll is to contribute to the spectral reflections of organisms. Noda et al. (2021) through modeling analyze that the seasonal variations in chlorophyll content of the species leads to a seasonal variation in the optical properties of leaf.

The data in Figure 7 demonstrates the spectrum responses of the ground based multiple samples of guava, cotton, mango, musambi, banana, kino, maize, and rice, recorded by using spectroradiometer. The reflectance of leaves exhibits a decrease in the visible range of the electromagnetic spectrum, specifically between 330 and 530 nm. Notably, there is a discernible fluctuation in reflectance, with a maximum value occurring about 400 nm, which

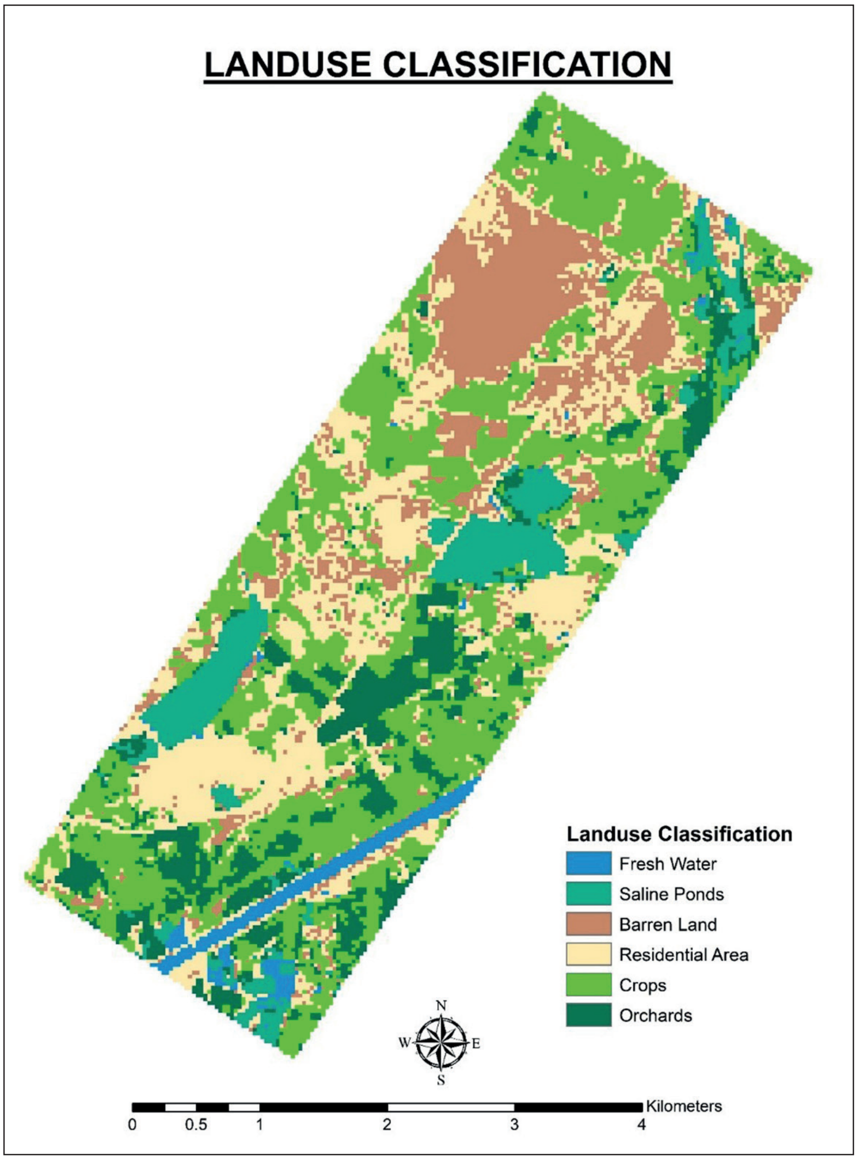

Figure 6. Land use classification

corresponds to the green region. The pigmentation of a plant determines the visible part of the spectrum in green plants. The infrared reflectance within the wavelength range of 600 nanometers. Tesfaye and Awoke (2021) indicated that different feature selection methods for the prediction of chlorophyll to find the best prototype model.

4. Conclusions and recomendations

There is a strong positive correlation observed between the SPAD values and the combined chlorophyll (a+b) content, as determined using regression analysis. The R^2 value in cereal crops and orchards exceeds 80%, providing evidence that the use of the SPAD nondestructive method is preferable

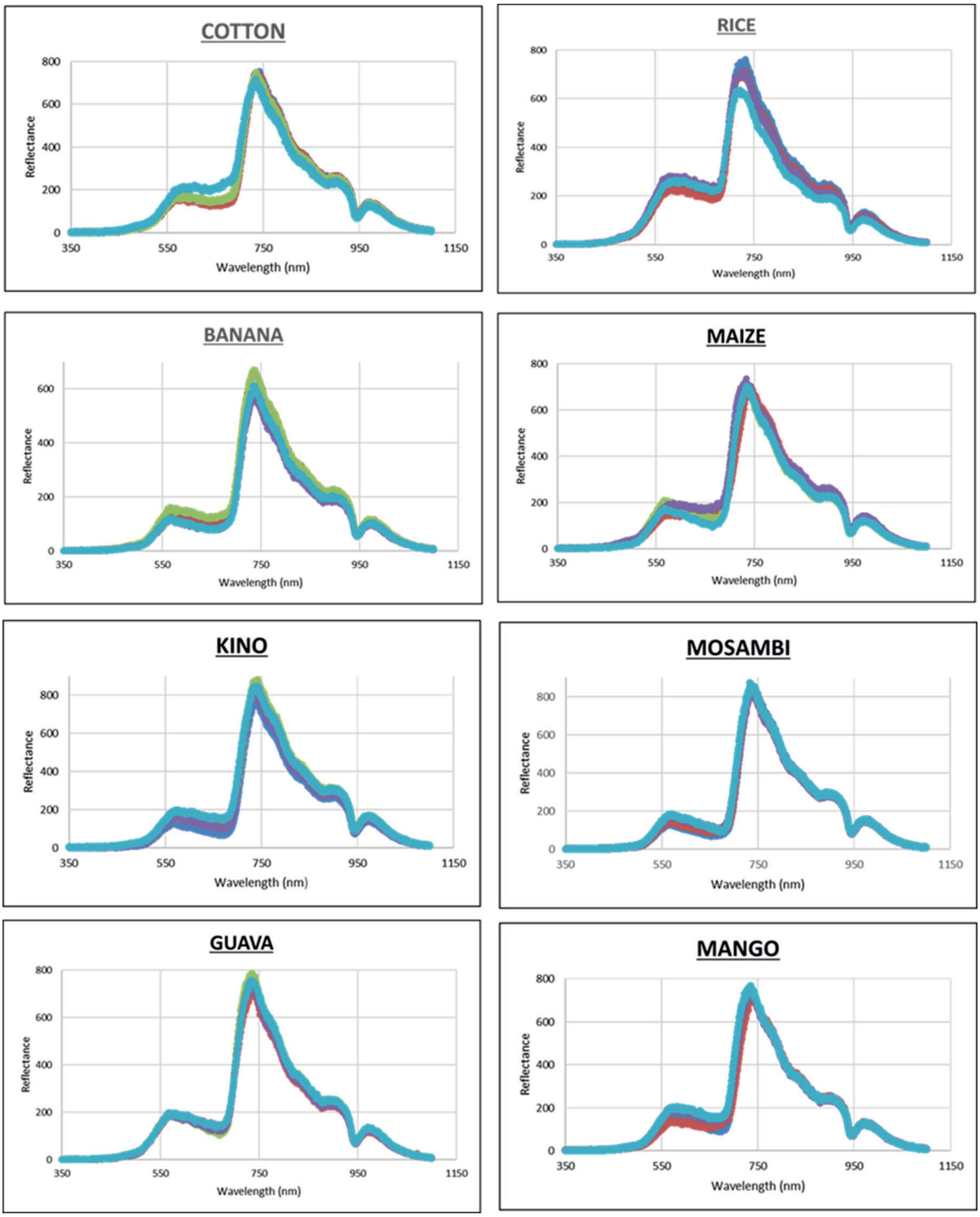

Figure 7. Spectral signature of orchards and crops

to the time-consuming non-destructive chlorophyll extraction method. The scatter diagram demonstrates a positive correlation between chlorophyll concentration and NDVI. The laboratory study of chlorophyll content and the utilization of satellite data to measure chlorophyll levels demonstrate a predominantly consistent pattern in agricultural crops. The reliability of satellite data for monitoring crops and orchards, specifically for the acquisition of chlorophyll content, has been determined to be a viable alternative to the labor-intensive procedures often conducted in laboratories. The utilization of Landsat-8, does not incur any financial expenses. The identification of crops and orchards can also be accomplished by examining the texture and canopy characteristics of trees.

A more extensive analysis might be conducted by accessing more advanced multispectral satellite data. The resolution of Landsat data is comparatively lower, specifically 30 meters, when compared to satellite data from spot, quick bird, planet, and sentinel. The study findings indicate a positive correlation between chlorophyll content and NDVI as observed in the scatter diagram. However, it is challenging to accurately identify individual crops or orchards using Landsat data. It is advisable to employ a high-resolution drone for the purpose of quickly visualizing and interpreting the canopy and texture of orchards and crops throughout all seasons of the year. This study provides an overview of the initial and fundamental ideas about the prediction of chlorophyll levels using multispectral data. The potentials of these methods are examined in relation to estimating chlorophyll content in crops and orchards within the specified study area. The chlorophyll concentration inside canopies exhibits temporal and spatial variations, necessitating a wide range of dynamic capabilities for chlorophyll evaluation in remote sensing approaches. This study has the potential to provide valuable insights for the advancement of precision agriculture practices, ultimately contributing to the enhancement and upliftment of the rural population in this region.

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