International Journal of Environment and Climate Change

Volume 13, Issue 9, Page 2602-2621, 2023; Article no.IJECC.104366
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Exploring the Potential of Proximal Remote Sensing in Plant Stress Phenotyping: A Comprehensive Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i92511

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/104366

Received: 29/05/2023
Accepted: 01/08/2023
Published: 02/08/2023

Review Article

ABSTRACT

The global challenge of feeding the world demands attention due to the projected population increase to 10.9 billion by 2050. Abiotic and biotic stressors, such as heat, drought, diseases, and pests, further compound the difficulties faced in achieving sufficient agricultural output. Early detection of crop stress is vital to mitigate yield loss and find appropriate agrotechnical solutions. However, the complex interactions between abiotic and biotic stressors and their impact on plant

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growth and yield present challenges in plant phenotyping and breeding. This review discusses recent advances in remote sensing technologies which offer promising solutions to overcome these challenges. Low-cost, reliable sensors and technologies facilitate data collection and interpretation, paving the way for proximal sensing and high-throughput phenotyping platforms. These automated platforms, equipped with imaging devices, enable non-destructive data collection and monitoring of plant properties over time. Optical methods like hyperspectral sensors, RGB imaging, remote sensing, and chlorophyll fluorescence contribute to the early identification of plant stress causes, facilitating the development of control strategies. By providing accurate and timely information on crop stress, these technologies offer essential support in enhancing agricultural productivity and ensuring food security for a growing global population.

Keywords: Remote sensing technologies; proximal sensing; high-throughput phenotyping; imaging devices; hyperspectral sensors; agricultural productivity; food security.

1. INTRODUCTION

Addressing the challenge presented in the 2nd Sustainable Development Goal (SDG) by Food and Agriculture Organisation [1], which pertains to nourishing the global population, stands out as one of the most formidable tasks confronting society in the present era. By 2050, it is anticipated that there will be 10.9 billion people on the planet. As a result, depending on the region, the food supply needs to be increased by 50% to 75% [2]. Due to short-term supply variations, climate change may endanger the stability of entire food systems [3,4]. On a regional scale, the potential consequences might not be immediately evident, but areas already vulnerable to hunger and malnutrition will probably experience a deterioration in food security due to the impact of climate change [4]. As a result, abiotic (such as heat and drought) and/or biotic (such as diseases and pests) conditions and their combinations will also increase in frequency [5], which will result in decreased agricultural output if management does not act quickly and effectively [6]. Early detection of crop stress is therefore crucial to be able to respond with appropriate agrotechnical solutions and thereby minimize yield loss before permanent damage occurs.

Crops often experience an increased number of abiotic and biotic stress combinations as a result of global warming and probable accompanying climate irregularities, which negatively impact their growth and yield [7,8]. Plant stress is characterized as a major departure from the ideal conditions for plant growth that may have detrimental consequences on the plant and restrict its ability to grow and develop normally. Almost every component of a plant can be affected by plant stress, though often only one or a few plant structures are affected, depending on the age of the plant and the source of the stress [9]. Crop plants experience abiotic stress and biotic stress, which are two different forms of environmental stress. Abiotic stress is responsible for significant crop losses globally and encompasses factors like radiation, salinity, floods, droughts, extreme temperatures, and heavy metal exposure. On the other hand, biotic stresses involve the invasion of various pathogens such as fungi, bacteria, oomycetes, nematodes, and herbivores that negatively impact crop health. [10]. Abiotic stresses, such as heat and drought, have been demonstrated to be more harmful to crop yield when they occur simultaneously than when they do so individually at various stages of crop growth [11,12]. In response to stress, plants experience several morphological, physiological, biochemical, and molecular changes that harm growth and productivity. Plant phenotyping is currently the main barrier to plant breeding and crop management. Obtaining large-scale plant phenotyping data quickly with high dimensionality, density, and accuracy from a single molecule to a whole organism is now the biggest problem. Although some bottlenecks have been greatly reduced by modern phenomics technology, several problems remain regarding how to accurately and quickly enhance throughput and accuracy while simultaneously defining and extracting complex features [13].

Many of these issues can be resolved because of recent advances in machine learning and remote sensing. As more and more low-cost, reliable sensors and technologies are used for data collection, storage, and interpretation, proximal sensing is in the early stages of potentially revolutionary development. [14]. Crop genetic improvement has been hampered by the lack of field-based high-throughput phenotyping methods [15,16], but recent reviews [17,18,19] have highlighted the opportunities now provided by sensor technology and the digital age. High-throughput phenotyping platforms have been created to automate data collection on numerous plants to facilitate the gathering of phenotypic measurements [20,21,22]. These platforms frequently have imaging devices installed, which collect data without causing any damage to the plant and track changes in plant properties over time [23].

Earlier, crop monitoring used to be done visually, either from the ground or occasionally from the air, by inspecting the crops. These days, there are optical methods available, including hyperspectral sensors, RGB imaging, remote sensing, and chlorophyll fluorescence, that can be used to identify plant stress causes early on, enabling the development of control methods and the minimization of damage brought on by stress [24]. Plants respond to severe biotic and abiotic circumstances by altering their physiology and metabolism through pulses of gene expression. To enhance agricultural development and increase yields, it became essential to identify and adopt novel technologies and approaches [25]. Mezera et al. [26] examined and compared the optical measurements and assessments of vegetation using both proximal and remote sensing techniques. The goal was to diagnose plant nutritional status on farms and implement site-specific crop management practices. In the last two decades, Proximal Remote Sensing has made unprecedented progress in Plant Phenomics (PP). It now enables observations across various scales, covering cellular to population-level studies, above-ground to underground assessments, and controlled indoor environments to field conditions [27]. Efficiently acquiring plant phenotyping parameters is crucial for modern agriculture. However, traditional manual methods have limitations in accuracy and efficiency. To overcome these challenges, researchers are turning to robotic platforms for eco-phenotyping. These platforms offer flexible movement and high automation, allowing for more targeted and ecologically relevant studies of plant phenotypes [28].

2. STRESS- DEFINITION AND PHYSIOLOGY

According to Jones and Jones [29], plants are sessile organisms subject to biotic and abiotic forces, or the physical, chemical, and biological environment defining their habitat. The success or survival of a plant species can be affected by both biotic and abiotic interactions. Plant physiologists have therefore focused on understanding the effects of various stress factors on plants [30], which is crucial for crop sciences (31). According to Lichtenthaler [32], plant stress is generally understood to be "any unfavorable condition or substance that affects or blocks a plant's metabolism, growth, or development." External factors that negatively impact plant growth, development, productivity rates, crop yields, etc. are referred to as stress in plants. Stress in plants typically results from certain abrupt alterations in the environment. However, exposure to a specific stress results in adaptation to that stress in stress-tolerant plant species in a time-dependent manner. [33]. However, some plants, such as desert plants (Ephemerals), may completely avoid stress [34].

The reaction is triggered by biochemical factors, such as phytohormones or enzymes, and it results in a cycle of damage and repair, activating the metabolism of the plant and, often, producing minor or imperceptible phenotypic adaptations (reversible and adaptive strain tolerance). "Phenotypic plasticity" is the term used to characterize a plant's capacity to alter the expression of its phenotype in response to environmental factors. According to Kranner et al. [35], the effects of a single stress factor, several stress factors, as well as the combination of biotic and abiotic stresses, can frequently cause very comparable physiological responses in plants. Additionally, the timing of stress effects must be taken into account. For instance, alterations in the flux rates of photosynthesis, respiration, and transpiration may be a sign of brief environmental changes [36]. Long-term stress that occurs during distinct developmental stages can have a variety of effects on growth. To avoid severe crop loss, detecting physiological changes in plants before they become apparent is essential [37].

When plants are exposed to biotic and abiotic stress, their metabolism is disrupted, implying physiological costs [38, 39, 40, and 41] and eventually lowering fitness and production. One of the most crucial aspects is abiotic stress, which has a significant impact on development and, as a result, causes significant losses in the field. In most plant species, the ensuing growth decreases can reach >50% [42]. Additionally, biotic stress is a problem that adds to the harm caused by pathogen or herbivore attacks and puts a lot of pressure on plants. Plants undergo long-term adaptations to change their phenotypic manifestations as the severity and length of the stress exposure grow. According to high or low light conditions, this may influence things like leaf size and thickness, stomatal density, or chloroplast function [43]. It should be emphasized that the effects of any stress factor typically lead to a complex interaction between the genes and environment of the plant, frequently resulting in various strains.

3. DIFFERENT FORMS OF PLANT STRESS AND THEIR COMMON SYMPTOMS

Multiple published reports have documented crop losses resulting from abiotic stresses [44]. In an ever-changing environment, various detrimental factors, including heat, cold, drought, and salinity, among others, can adversely impact agricultural land and crop productivity. Heat stress refers to prolonged exposure to temperatures beyond a critical threshold, causing irreparable harm to plant well-being [45]. It disrupts plant cell balance, hindering growth and potentially leading to plant mortality [46]. This poses a significant global threat to crop production [47]. Similarly, cold temperatures also impose limitations on the growing seasons of numerous plant species. Chilling stress, apart from causing noticeable phenotypic changes, also leads to significant biochemical and physiological alterations [48, 49]. Drought, a major environmental stress, has a severe negative impact on crop yield [50].

Key effects of drought include poor germination [51, 52], reduced nutrient availability and photosynthesis [53], decreased leaf number and size [54], and reduced fresh and dry weight of plants [55]. The plant's response to drought is influenced by factors such as growth stages, plant species, age, and the severity and duration of the drought stress [56]. Salinity is recognized as a highly detrimental environmental stress that significantly diminishes crop productivity and quality worldwide [57]. It affects plant growth and development, increases intracellular osmotic pressure, and can lead to toxic levels of sodium accumulation. Salinity stress induces various symptoms that resemble those caused by drought stress. The presence of excessive salt in the soil impairs the plant's water absorption capability, leading to a reduction in its growth rate [58]. Heavy metal stress inactivates enzymes and disrupts essential metallic ion substitution reactions, affecting membrane integrity, photosynthesis, and respiration [59]. Heavy metals induce oxidative stress by stimulating the production of hydrogen peroxide (H2O2), superoxide radicals (O− 2), and hydroxyl radicals (OH) [60]. Excessive ultraviolet (UV) sunlight exposure disrupts plant growth, leading to ROS overproduction and potential cellular imbalance [61]. Waterlogging alters soil properties, causing hypoxia or anoxia, significantly reducing plant growth and survival [62].

Plants face the risk of infection by various pathogens, such as bacteria, fungi, viruses, and nematodes, along with attacks by herbivorous pests [63]. Biotic agents directly induce plant responses; for instance, herbivory results in immediate consequences like water loss, reduction in leaf area, and necrosis [64]. Biotic pathogens initiate local effects and trigger systemic plant responses, including signal pathways reducing stomatal conductance [65] and increased production of volatile organic compounds (VOCs) as defense mechanisms [66]. Fungal pathogens exhibit diverse effects. Some pathogens initially stimulate photosynthesis, benefiting from increased sugar production during early infection stages [67]. However, in later stages, they can decrease photosynthetic capacity, leading to visible foliar lesions [68]. This fungal diversity gives rise to various plant diseases such as anthracnose, leaf spot, rust, or wilt [69]. These diseases can manifest as changes in the plant canopy's optical reflectance properties, detectable through radiometric changes or computer vision approaches for disease classification at the leaf level [70].

4. PROXIMAL REMOTE SENSING TECHNIQUES FOR STRESS PHENOTYPING

Understanding stress-related phenotypic traits and their interrelationships requires precise knowledge of phenotyping in both controlled and field conditions. Advanced techniques, such as visible light imaging, hyperspectral imaging, infrared imaging, fluorescence imaging, and X-ray computed tomography, enable the non-destructive acquisition of plant phenotype data using visible to near-infrared light sources [72]. These imaging-based, high-throughput platforms integrated with advanced software systems are valuable tools in plant biology [73]. By focusing on the interaction between light and plants, these

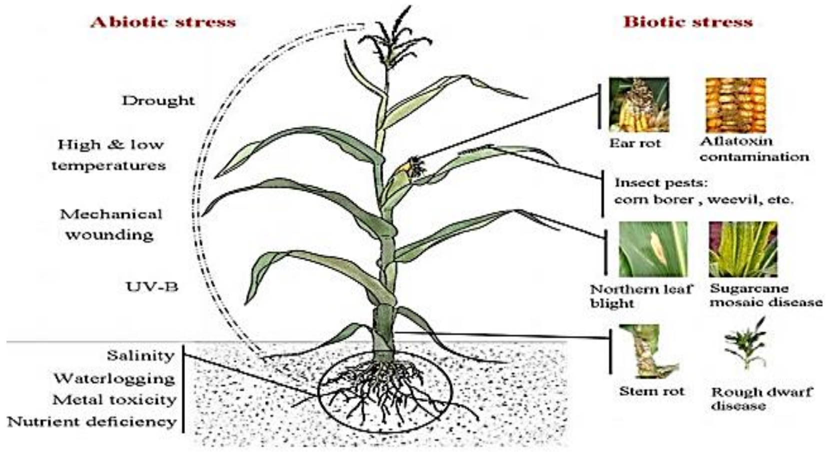

Fig. 1. An overview of major abiotic and biotic stresses.Taken from Umar et al. [71]

imaging techniques provide accurate measurements of quantitative phenotypic traits. Recent imaging technology advancements have enabled the non-destructive collection of physiological data over time, facilitating the analysis of complex plant responses in measurable traits. Image-based technologies have proven valuable in quantifying crop responses to stress, both in controlled environments and field trials [74]. Implementing high-dimensional phenotyping requires uniform experimental protocols, calibrated imaging sensors, and precise data analysis. The following section outlines the imaging devices currently used for high-throughput phenotyping of crop plants.

4.1 RGB Imaging

To measure the morphological characteristics of plants, the RGB camera approach is the most extensively used system [75]. This is because it is both affordable and simple to deploy. In contrast to consumer cameras, RGB cameras have an infrared blocking filter (VIS camera), which only picks up light with a wavelength between 400 and 700 nm. To determine the color of each pixel, the VIS camera uses red, green, and blue color sensors. It is possible to collect morphological or color data by using the pixel values of plants that the image processing system has detected [76]. To minimize variables and enable an in-depth examination of each plant's response to stress, fixed equipment is employed in laboratory research. These studies also allow for the capture of picture data throughout the experiment and the detailed examination of how each plant reacts to stress. Recently, visible light-based imaging systems have gained attention due to their affordability, simplicity of operation, and ease of maintenance. Two-dimensional (2D) digital pictures captured using visible light are utilized for quantifying various attributes such as shoot-related features, leaf architecture, shoot elongation, as well as seed and root morphological characteristics [77].

Ge et al. [78] extracted plant pixels from RGB images to correlate with shoot fresh weight, dry weight, and leaf area. Neilson et al. [79] estimated plant biomass and compared it with actual plant size. Positive correlations were found between leaf area and shoot biomass in a water-limiting experiment. Leaf greenness was estimated using the RGB to HIS color system. Field studies use sensors attached to vehicles or aircraft for large-scale analysis. UAV-based RGB sensors provide high-resolution color information for vegetation indices. Bhandari et al. [80] and Francesconi et al. [81] computed canopy features using UAVs for monitoring drought effects on wheat. RGB cameras on fixed facilities reduce data errors from vehicle movement. Becker and Schmidhaler [82] used an RGB sensor in a drought treatment facility to estimate yield and growth rate correlation. RGB images enable pixel-level analysis of biomass changes under drought stress. Biomass inference using pixel counts correlates well with various crops [83]. RGB images can predict plant growth changes and analyze specific parts. Color analysis detects leaf wilting and chlorophyll deficiency due to drought stress [84]. RGB sensors on UAVs provide limited upper images for vegetation index analysis. Phenotypic information helps identify candidate genes for specific traits Campbell et al. [83] used the HTP platform to quantify daily shoot biomass and soil water content and simulate shoot growth. Han et al. [85] examined the relationship between RGB-based vegetative indexes (VIs) and biomass traits in 20 varieties of kenaf using UAV-captured RGB images. Positive correlations were found between VIs and stem diameter, node number, individual surface area, and estimated plant height. VIs proved valuable in predicting kenaf biomass. Notably, correlations differed between early and late growth stages. Following that, a genome-enabled growth model was combined to identify several candidate genes. Using digital image processing, a multi-plant multi-disease detection system was developed in [86], where disease identification was carried out using color transformations, color histograms, and a pairwise-based classification system. A smartphone-based image processing software [87] for diagnosing plant diseases, where the created system was tested for diseases in vineyards using images of grape leaves. Through thresholding of the RGB pixel values, the analysis begins by separating region lesions from the background and healthy tissue. Spot count, grey level, and area were among the traits that were extracted, along with color histograms. 90% of probable diseases were successfully identified by the application.

4.2 Near-infrared Imaging

The green parts of plants consistently had the maximum reflectivity at near-infrared wavelengths between 700 and 1400 nm in many studies, While unhealthy plants reflect more red light than healthy plants, soil reflects very little near-infrared light. Additionally, freshly absorbed water by leaves scatters near-infrared wavelengths [88]. To confirm how plants respond to drought stress, near-infrared imaging (NIR) is used [89]. These infrared imaging systems offer large-field images with excellent spatial resolution and accurate measurements while simultaneously operating in a variety of environmental situations [90].

The plant water content estimated by NIR is also used to identify candidate gene regions. El-Hendawy [91] et al. used a combination of the absorption rate is maximum in the spectral region between 1400 and 1450 nm, and it is strongly associated with the moisture content of plants. With the aid of a partial least squares regression (PLSR) model and a NIR picture [92] were able to locate water absorption bands. NIR can offer a reliable method for non-intrusive, real-time monitoring of the water content of leaves on crop plants. Using NIR imaging, Chen et al. [93] looked into the dynamics of water content and the drought responses of 18 distinct barley cultivars. After experiencing drought stress, plants had a quick decline in NIR signal, which recovered after re-watering.

This technology may immediately identify drought stress in plants since it uses NIR to measure reflectance in a particular wavelength band, which permits a speedy study of plant water content. Additionally, by combining NIR imaging with visible imaging, or visible to short-wave infrared (VSWIR; 0.4-2.5 m), this method offers deeper insight into plant health under various stress conditions because it provides well-defined spectral features for pigments, leaf water content, and biochemicals like lignin and cellulose. Further, infrared thermography has also been used to study stomatal responses under salinity and drought by visualizing differences in canopy temperature [94].

4.3 Hyperspectral Imaging

Hyperspectral sensors capture numerous bands per pixel across the visible, NIR, and SWIR regions [95]. While the data complexity requires advanced computational resources and storage capacity, high-resolution images with narrow spatial coverage can differentiate responses to different stresses. Hyperspectral imaging enables the detection of early drought stress symptoms, imperceptible to the naked eye. By employing suitable spectral analysis techniques, plant responses to drought stress can be easily evaluated. In laboratory settings, stationary facilities can generate normalized difference vegetation index (NDVI) values by utilizing different hyperspectral wavelength ranges that are segmented based on plant structure. In recent times, hyperspectral sensing has become a highly promising technology for evaluating plant physiology and their responses to stress. This is achieved by combining both spatial and spectral information. The two primary categories of hyperspectral sensors are imaging sensors and non-imaging sensors [96].

While imaging hyperspectral sensors combine spectral and spatial resolutions, non-imaging hyperspectral sensors just detect the average spectral information over the area under study [97]. The visible (400–700 nm), near-infrared (700–1000 nm), and short-wave infrared (1000–2500 nm) wavelength ranges were the subject of earlier experiments using hyperspectral sensors [98]. Since plants usually reduce the concentration of leaf chlorophyll, reflectance in the visible range can be correlated to leaf pigment content [99], whereas the near-infrared range is primarily influenced by leaf structure (such as leaf trichome density or leaf thickness) and leaf water content [100]. Apart from canopy cover information, vegetation indices, including the red-edge position, also offer insights into leaf nitrogen or chlorophyll content. Due to this capability, these indices are extensively utilized for assessing canopy nitrogen content in crop management. Commercial sensors designed for mounting on tractor booms are commonly employed for this purpose.

The chemical compositions of the leaf, such as lignin or cellulose [101], have an impact on the short-wave infrared range in addition to the leaf's water content. Plant phenotyping has not yet utilized the ultraviolet spectrum (200-380 nm). Many plant compounds, including flavonoids, amino acids, anthocyanins, and nucleosides, have UV-range absorption maxima [102, 103]. This method performs well for determining the water content and canopy reflectance of each plant section. Ge et al. [104] divided the plant into stems and leaves using different wavelengths and employed hyperspectroscopy to calculate the NDVI of maize. [105,106] have used hyperspectral data to compute various spectral indices and look for relationships with grain yield and drought stress. Specific vegetation indexes can be identified by extracting appropriate wavelengths from hyperspectral data [107,108]. Additionally, for genomic estimated breeding, hyperspectral data have been utilized in conjunction with molecular markers to improve projections of grain yield [109]. Utilizing the vegetation index, which is defined as a linear combination or ratio of reflectance at many single wavelengths, is a standard method for the hyperspectral-based estimate of plant characteristics under drought stress. There are differences in the photosynthetic apparatus, water content, plant organs, and yield among the physiological and biochemical responses of vegetation to drought stress. In a nutshell, hyperspectral imaging can use different vegetable indices to analyze changes in plants under drought stress. Optical images can provide normalized differential vegetation index (NDVI) and soil-adjusted vegetation index (SAVI) data, enabling the diagnosis of water stress and soil moisture conditions in various crops [110]. Vegetation indices, including the red-edge position, also offer insights into leaf nitrogen or chlorophyll content. Due to this capability, these indices are extensively utilized for assessing canopy nitrogen content in crop management. Commercial sensors designed for mounting on tractor booms are commonly employed for this purpose [111].

HS imaging is a promising field for pre-symptomatic crop health monitoring. In [112], an early detection system for tobacco mosaic virus (TMV) used HS imaging in the Visible /NIR spectral range (380 nm to 1023 nm). Spectral and textural features at selected wavelengths enabled a four-class classification (healthy, 2 DAI, 4 DAI, and 6 DAI) using machine-learning-based classifiers and the successive projections algorithm (SPA). Similarly, in [113], an HS imaging system detected tomato spotted wilt virus (TSWV) disease in capsicum plants with over 90% accuracy using support vector machine (SVM), classifiers on three types of features: full spectrum, spectral vegetative indices (VIs), and data-driven probabilistic topic model-generated features from both Visible -NIR and Short Wave -NIR hypercubes.

4.4 Fluorescence Imaging

Fluorescence imaging is employed to estimate the rate of photosynthesis and monitor the impact of plant pathogens [123,124] as well as to detect early stress responses to both abiotic and biotic factors before any decline in growth becomes measurable [125, 126, 127, 128]. In this imaging technique, blue wavelength light with a wavelength of less than 500 nm is directed at the plants, causing them to emit fluorescence light in the red region of the spectrum, specifically at 600-750 nm. These differences in fluorescence are captured through photography and converted into false-color signals using computer software for further analysis [129]. The primary impact of abiotic stresses is on the chlorophyll content, making chlorophyll fluorescence a commonly used tool in phenomics to observe the effects of various environmental factors on genes and the plant's capacity to sustain photosynthesis under such conditions [129]. By measuring chlorophyll fluorescence, which refers to the emission of excessive energy by the plant in the form of fluorescence, fluorescence sensors can evaluate the photosynthetic efficiency of the crop being examined [130]. Visible or ultraviolet (UV) light is used to stimulate the plant, and the resulting fluorescence is captured using charge-coupled device (CCD) cameras [131].

When plants are exposed to UV light in the 340-360 nm range, two types of fluorescence are produced: red + far-red fluorescence and blue-green fluorescence. A multicolor fluorescence imaging principle is utilized to collect fluorescence emitted in four spectral bands, each represented by a specific wavelength—blue (F440), green (F520), red (F690), and near-infrared (F740) [132]. Fluorescence and chlorophyll content serve as crucial indicators of the metabolic states of plants. Additionally, fluorescence imaging can be utilized to study various aspects such as stomatal movement (133), phloem loading and unloading [134], the correlation between spatiotemporal variation of photosynthesis and growth limitation [135], and plant metabolite content [136] under stressful conditions [137,138,139]. Fluorescence imaging has been employed for different purposes, including stress detection at the primary level [140,141,142] and the characterization of heterogeneity in leaf photosynthetic performance [143]. McAusland et al. [144] proposed an experimental method for observing photosynthesis and photoprotection in crops across diverse light environments through fluorescence analysis. Similarly, in field conditions, the photosynthetic efficiency of a target plant can be assessed by measuring its chlorophyll fluorescence using a portable fluorescence meter in environments with drought stress or adequate watering [145, 146]. Chlorophyll fluorescence is useful for monitoring linear electron transport, which is closely linked to CO2 uptake during photosynthesis [147]. Ahlam et al. [148] successfully applied a method to investigate both abiotic stress (drought and salt) and biotic stress (Powdery mildew). The method proved effective for abiotic stress but revealed the need for a spatial resolution to tackle the point-wise spread of Powdery mildew infection. Cannière et al. [149] studied the impact of drought stress on vegetation using sun-induced chlorophyll fluorescence (SIF) observations. The findings suggest that SIF and reflectance-based indices provide complementary information for monitoring vegetation stress. The study highlights the potential of SIF data from the upcoming FLuorescence EXplorer (FLEX) satellite to assess plant water status.

Several studies have focused on ChlF-based imaging, which includes the detection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) in sweet potatoes using thermal imaging and ChlF [150]. This study highlighted the operating efficiency of PS-II and photochemical quenching as the most sensitive parameters for quantifying virus effects, surpassing measures like maximum quantum efficiency, non-photochemical quenching, and leaf temperature. Additionally, an early detection system was developed by combining HS- and ChlF-imaging [151]. By integrating both techniques into one device, the classification errors were reduced to less than 5%, leading to more accurate results.

4.5 Thermal Imaging

Thermal imaging is used to measure leaf surface temperatures to study plant water relations, specifically for stomatal conductance because a major determinant of the leaf temperature is the rate of evaporation or transpiration from a leaf. Abiotic or biotic stresses often result in decreased rates of photosynthesis and transpiration [152,153]; and, the remote sensing of the leaf temperature by thermal imaging can be a reliable way to detect changes in the physiological status of plants in response to different biotic and/or abiotic stresses. This method uses radiation emitted by an object to generate an image, which increases as the temperature of the object increases above absolute zero. Thermal sensors can use visualized image data to detect changes in plant temperature caused by transpiration due to stomatal closure. Therefore, thermal imaging can measure temperature-related features such as water content, transpiration rate, and stomatal conductance through model-based estimation [154]. The canopy temperature has been used successfully in breeding programs for drought-prone environments. In plant phenotyping, thermal imaging offers canopy temperatures to detect differences in stomatal conductance as a measure of the plant response to the water status and transpiration rate [155, 156], both in the field and in the greenhouse. Canopy temperature differences were compared with the surrounding air (for example, the canopy temperature depression, or CTD) as measured by thermal infrared imaging, and these results have been used as a selection criterion in breeding programs for drought resistance [157]. In addition, thermal imaging has been used for many crops, from small cereal grains to maize [158] and fruit trees [159]. It has also been used in combination with spectral imaging for the enhanced estimation of leaf water content [160.]. Romano et al. [161] used thermal imaging in maize under reproductive stage drought stress and identified its potential for accelerating phenotyping and screening in maize water stress breeding programs. Estimating stomatal conductance and water status through leaf-level thermal imagery methods could provide valuable information on the effects of non-uniform stomatal responses on photosynthetic rates [162].

A field experiment using a thermal sensor analyzed temperature differences between plants and plots under drought stress. Significant correlations were found between seed yield and canopy temperature, confirming the impact of drought stress on biomass and yield. Relevant genomic regions and extreme genotypes for canopy temperature were identified [163,164]. Aerial thermal infrared image analysis assessed canopy temperature and association mapping identified 52 SNPs significantly associated with canopy temperature. Canopy temperature was used to estimate the crop water stress index (CWSI), an indicator for selecting drought-resistant varieties [165]. Thermal imaging provides valuable information on stomatal conductance, allowing quick evaluation of temperature increases and assessment of changes in plant transpiration rates and drought stress levels. Its use, along with thermal cameras, improves crop phenotyping for drought adaptation [164,166].

Thermal imaging rapidly diagnosed crop diseases [167]. For tomato mosaic disease and wheat leaf rust, the maximum temperature difference (MTD) ranged from 0.2°C to 1.7°C and 0.4°C to 2°C, respectively. MTD increased as the disease progressed, detectable 5 to 7 days before visible symptoms. In apples, IR thermography sensed scab disease caused by *Venturia inequali*ty [168]. MTD linearly correlated with infection size (R2 = 0.85), later decreasing due to leaf senescence.

4.6 Non-Imaging Spectroscopic Methods

Non-imaging spectroscopic methods comprise VIS/IR reflectance and transmittance spectroscopy, along with Raman spectroscopy. VIS/IR spectroscopy is a type of hyperspectral imaging that records only spectral information (not spatial/pixel data) within the VIS/IR wavelength range. Plant health is assessed by measuring either the reflectance of the leaf/tissue surface or the transmitted light through the leaf tissue and correlating it with relevant indicators. A VIS-NIR reflectance spectroscopy system [169] for detecting HLB or citrus greening disease in citrus trees used two portable halogen lamps and a field-portable SVC HR 1024 spectroradiometer. The system collected reflectance data from 350 nm to 2500 nm (989 data points) and included a laser pointer for target area designation. The mobile platform allowed in-field operations, and the best-performing classification algorithm was quadratic discriminant analysis (QDA), with a 95% average accuracy. Multiple recent studies have used spectroscopic methods for plant disease detection. For instance, research has been conducted into the early detection of potato late blight using leaf reflectance measurements with a spectroradiometer [170]. Another study applied NIR spectroscopy for the detection of bitter pit disorder in honey crisp apples, recording spectra for 40 apples at three different times post-harvest [171]. Non-imaging VIS/IR spectroscopy (VIS/IR-spec) has unique advantages, such as a simpler and cheaper setup involving a light source and a spectroradiometer, compared to hyperspectral (HS) imaging. Data analysis is less complex since image pre-processing is unnecessary. However, VIS/IR-spec's lack of spatial information complicates its field application due to potential environmental interference. Raman spectroscopy (RS) is another analytical spectroscopic technique that reveals molecular structure information by analyzing molecular vibrations from inelastic photon collisions. A recent study explored the use of a handheld Raman spectrometer to detect and identify fungal pathogens in maize kernels [172].

4.7 Other Imaging Techniques

Functional imaging and optical 3D structural tomography are two recent technological developments that have shifted more and more towards in vivo live imaging of plants. Functional imaging focuses on physiological changes to assess photosynthetic performance under stress, such as ChlF imaging and positron emission tomography (PET). Positron emission tomography (PET) is an imaging technique that quantitatively and non-destructively assesses the 3D spatial distribution and kinetics of radio-tagged biomolecules in a living subject. The elements required for plant growth are 11C, 13N, and 15O, which are often utilized positron-emitting radionuclides [173]. The transport of 11C-labeled photo-assimilates can be repeatedly observed in 3D by PET when CO2 is consumed during photosynthesis [174].

Table 1. The most commonly utilized spectral indices for crop stress monitoring include ρRED, ρGREEN, and ρBLUE, which represent the spectral reflectance of the red, green, and blue bands, respectively. Additionally, ρNIR stands for the reflectance of the near-infrared band and ρSWIR refers to the reflectance of the shortwave-infrared band. These indices play a crucial role in assessing and tracking crop stress levels

Name	Abbreviation	Formula	Description with related traits and challenges	References
normalized difference vegetation index	NDVI	(ρNIR – ρRED)/(ρNIR + ρRED)	Assess green vegetation using a normalized ratio within the range of −1 to 1.	[114]
normalized difference water index	NDWI	ρNIR − ρSWIR)/(ρNIR + ρSWIR)	Quantifies variations in leaf water content by utilizing Near-Infrared (NIR) and Shortwave Infrared (SWIR) spectral bands.	[115]
difference vegetation Index Jordan	DVI	NIR – Red	Highly responsive to vegetation quantity; characterized by its simplicity as a ratio; however, it does not account for discrepancies between reflectance and radiance due to atmospheric conditions or shadows.	[116]
green normalized difference vegetation index	GNDVI	(ρNIR − ρGREEN)/(ρNIR + ρGREEN)	An adaptation of NDVI (Normalized Difference Vegetation Index) with increased sensitivity to chlorophyll content.	[117]
photochemical reflectance index	PRINT	(ρ531 − ρ570)/(ρ531 + ρ570)	Serves as an indicator of leaf and plant canopy photosynthetic efficiency.	[118]
structure insensitive pigment index	SIPI	(ρ800 − ρ445)/(ρ800 + ρ680)	Acts as an indicator of increased canopy stress, particularly related to carotenoid pigment levels.	[119]
moisture stress index	MSI	(ρ1599)/(ρ819)	Highly responsive to rising leaf water content and widely utilized for canopy stress analysis and productivity prediction.	[120]
Leaf water content index Ceccato et al. [144]	LWCI	log(1 − (ρNIR − ρMIDIR))/ −log(1 − (ρNIR − ρMIDIR))	Assesses the moisture content of the leaf canopy.	[121]
modified red edge NDVI Sims & Gamon [35]	mRENDVI	(ρ750 − ρ705)/(ρ750 + ρ705 −2 * ρ445)	Leverages the sensitivity of the vegetation red-edge to detect subtle changes in canopy foliage content, gap fraction, and senescence.	[122]

Table 2. Applications and limitations of common sensors used for proximal remote sensing

Sensor Type	Applications	Limitations
RGB Cameras	Used for imaging canopy cover and canopy color. They provide insights into chlorophyll concentration using greenness indices and enable canopy architecture estimation with 3D stereo reconstruction.	Lack of spectral calibration, leading to relative measurements. Susceptible to shadows and changes in light conditions, hindering automated image processing.
Spectral sensors	Assess the biochemical composition of leaves and canopies, including pigment concentration and water content. Contribute to canopy architecture assessment with indices like NDVI.	Require frequent calibration and susceptible to changes in light conditions, necessitating white reference calibration. Canopy structure and camera/sun geometries affect measurements, posing data management challenges.
Fluorescence	Suitable for assessing the photosynthetic status of plants and indirectly measuring biotic and abiotic stress factors.	Field-level canopy fluorescence measurements can be challenging due to the low signal-to-noise ratio, but techniques like laser-induced fluorescence transients (LIFT) and solar-induced fluorescence extend the range for remote sensing.
Thermal sensors	Employed to measure stomatal conductance and detect water stress induced by biotic or abiotic factors.	Changes in ambient conditions cause canopy temperature fluctuations, making comparisons over time difficult without references. Distinguishing soil temperature from plant temperature in sparse canopies is problematic, hindering automated image processing. Calibration and atmospheric correction are often required for accurate results.
X-ray Computed Tomography (CT)	It provides high-resolution, three-dimensional architecture	low automation and low throughput, high cost
Magnetic Resonance Imaging (MRI)	Gives information about water status, transportation, and root architecture, Provides three-dimensional architecture	low throughput and high cost
Positron Emission Tomography (PET)	Gives information about the translocation and transport of elements, shows the movement and path of positron through the plant	low throughput, high cost

Nuclear magnetic resonance is used in nuclear magnetic resonance imaging (MRI), which produces images and recognizes nuclear resonance signals coming from 1H, 13C, 14N, and 15N. In seeds [175], whole root systems growing in or near natural soil [176], and entire plants, 3D datasets of plant structures can be acquired using an MRI. It may then assess water spread and movement through the xylem and phloem in crops such as castor beans, tomatoes, tobacco, and poplars [177]. It is possible to monitor dynamic changes in plant structure and function by combining MRI and PET technology.

X-ray CT is a computer-processed X-ray technique that can create 3D representations of an object's internal structures from a collection of 2D radiographic images taken around a single axis of rotation. It has been effectively applied for a variety of purposes, including the analysis of soil structural heterogeneity [178] and the visualization of plant structures [179]. The costs and duration of the scans are the limitations of CT, though.

With the help of fluorescent sensors that are genetically programmed, Forster resonance energy transfer (FRET) is a cutting-edge non-invasive technique for high-resolution imaging of tiny molecules in live tissue. It has been successfully utilized to detect calcium and zinc dynamics in roots during sugar transport [180]. It permits the discovery of numerous paths and dynamic processes of the target molecule. FRET is a superb tool for advanced phenotyping that can solve several fundamental queries about plant growth and development. Wheat plants were phenotyped under both control and salt stress conditions using the 3D laser scanner Plant Eye. From the overhead data cloud, the system calculated characteristics including 3D leaf area, plant height, and leaf number. In wheat under salt stress, correlations between the manually measured characteristics (leaf area, fresh and dry biomass) and the Plant Eye-scanned trait (3D leaf area) were observed [181]. Other sensors may be able to offer 3D structural data. Liar is conceivably the most commonly utilized type of sensor for 3D canopy reconstruction [182,183]. Such laser devices have been utilized to determine the plant area density profiles of a wheat canopy and for quick LAI mapping [184,185].

5. CONCLUSION

In conclusion, proximal remote sensing offers a powerful and effective approach to plant stress phenotyping. The ability to collect real-time, accurate, and non-destructive data can significantly contribute to our understanding of plant health, leading to improved agricultural practices, increased crop resilience, and better food security. To fully leverage the potential of proximal remote sensing, ongoing research, technological advancements, and collaborative efforts between scientists, farmers, and technology providers are crucial. While proximal remote sensing has shown great promise, there are still challenges and limitations to address. These may include the need for specialized equipment, data processing complexity, and potential interference from environmental factors. Additionally, access to data and affordability can be barriers in some regions.

ACKNOWLEDGEMENT

I express my gratitude to the National Agriculture Higher Education Project (NAHEP) and the Centre for Advanced Agricultural Science and Technology (CAAST) for their valuable assistance in skill development related to the utilization of spatial data for managing natural resources in agriculture. Additionally, I am thankful to the Department of Soil and Water Engineering at JNKVV, Jabalpur, for their financial and other forms of support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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