Chapter 15
Remote Sensing Algorithms and Their Applications in Plant Phenotyping

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Abstract Assessing phenotypic traits associated with physiology, biochemistry, and plant health based on leaf spectral reflectance properties has become an important high throughput tool in agriculture research. Precise quantification and monitoring of plant responses to stresses (abiotic or biotic) help researchers' phenotype different genetic resources, map genetic loci, and choose donors for trait development. Studies have shown the potential use of leaf hyperspectral reflectance in assessing the plant phenotype under biotic and abiotic stress conditions. We compiled wavebands or reflectance strongly related to the lab and field-based measurements for pigments, leaf nitrogen, and leaf water content. This chapter also highlights the recent applications of hyperspectral reflectance in plant phenotyping, stress diagnosis, species classification, and robust statistical methods. Furthermore, we discuss the need for advanced analytical tools and their potential applications in plant phenotyping.

Keywords Electromagnetic spectrum · Diagnosis · Hyperspectral reflectance · Pigments · Leaf nitrogen · Phenotyping · Remote sensing · Sensors

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15.1   Introduction

Based on evolutionary history, genetic background, and stress adaptation capacity, plants readily express differences in physiology and biochemistry, which can be detected using leaf or canopy spectral reflectance changes (Roitsch et al. 2019; Grzybowski et al. 2021). Along with the differences in morphology or ideotype at the plant level, plants have also evolved to synthesize different combinations of leaf pigments and other compounds to support growth and development under various environmental conditions. Improved detection and monitoring of plant health have been vital areas of research under a rapidly changing climate. Needless to say that high throughput non-invasive sensing-based phenotyping is faster, cheaper, reliable, unbiased, extendable, and robust.

Remote sensing techniques have been utilizing leaf optical properties (reflectance, absorbance, and transmittance) to understand leaf pigments (visible range), cell structure (near-infrared), and biochemical properties (shortwaves) using the leaf or canopy signatures. When a leaf or canopy is exposed to electromagnetic (EM) radiation, the *reflected radiation* can be measured and recorded as *reflectance spectrum.* Sensor modalities to study reflectance can be grouped into two categories (based on the signal type): optical and EM. Other choices include acoustic (ultrasound), magnetic (MRI), X-ray, thermal, electronic (electron-microscope observations), and mechanical sensors. To measure object (e.g., leaf or canopy) properties, most light/sound-based signal processing sensors/algorithms rely on the signal frequency, and the transduction depends on the following:

- Amplitude of the input/reflected/refracted signal
- Directionality of the input/reflected/refracted signal
- Time-of-flight measurement of the received signal

Let us recap a *(conveniently biased)* sampling of definitions from image processing. An image is the numerical representation of any real-world picture; it is a multi-dimensional array (or matrix) of numbers, each representing the amount of signal intensity sampled in a physical region, known as the *pixel.* There are a few choices to both describe the relative intensities of the different colors at each pixel (color space) and store images/parameters (Gonzalez and Woods 2018). Object-light interaction is dependent on both the incident signal characteristics (power level, frequency, bandwidth, etc.) and the structure and chemical constituents of the object. The signal's frequency is invariant as it travels through a medium. Thus, the velocity (and wavelength) of light traveling from vacuum through a non-vacuum medium will decrease. The ratio of light velocities between the medium and vacuum is the *medium's refractive index (RI).* Characterizing the object's structure based on the reflection/refraction signal can be found in more detail in text describing Snell's law or Fermat's least time principle.

Recall the wavelength ranges of the EM spectrum (Fig. 15.1). The EM spectrum is divided into four major wavelengths (λ) bands: visible (350–700 nm), near-infrared (NIR, 700–2500 nm), mid-infrared (MIR, 2500–25,000 nm), and
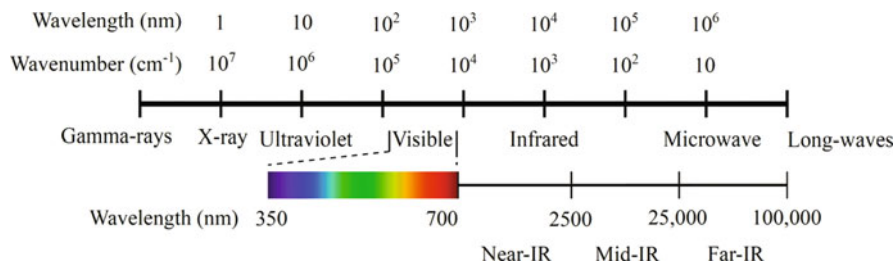
Fig. 15.1 Electromagnetic spectrum

far-infrared (FIR, 25,000–100,000 nm) (Lin et al. 2004; Manley 2014). The EM wave (light/IR) penetrates the surface of the specimen, and its penetration depth is defined as the depth at which the incident light intensity is reduced by 99%. It has been observed (Lammertyn et al. 2000; Qi et al. 2010) that the penetration depth is inversely proportional to the wavelength, which implies that the IR signal can penetrate deeper into the cell structure.

When EM strikes on the matter or canopy, three interactions can occur: *absorb*, *reflect*, and *transmit*. In plants, the EM absorbed can be used for photosynthesis, converted to heat, and re-emitted as fluorescence (van Bezouw et al. 2019). The leaf surface can reflect part of the incident EM radiation, which can ultimately reach an observer to perceive as color. The remaining portion of the incident radiation can transmit through the leaf and leave from the other side. All these interactions are related to each other and can be denoted as in Eq. (15.1)

$$I_\lambda = A_\lambda + R_\lambda + T_\lambda, \tag{15.1}$$

where I_λ is the incident radiation energy, A_λ is the absorbed energy, R_λ is the reflected energy, and T_λ is the transmitted energy at wavelength λ.

As Eq. (15.1) indicates, all these phenomena are wavelength-dependent since the absorbance depends on the properties of the leaf, more specifically, different chemical compounds or bonds present in the leaf. Chemical bonds absorb EM photons at specific wavelengths causing vibrational and rotational changes in the molecules (Türker-Kaya and Huck 2017). These absorbances at different wavelengths, called spectral signatures or fingerprints, can effectively estimate the leaf's other compounds or related properties. For example, the leaf nitrogen (N) and chlorophyll molecules have spectral signatures at 650 and 940 nm, which are used to estimate leaf nitrogen and chlorophyll contents (Wood 1993; Blackmer et al. 1994; Daughtry et al. 2000). Most fundamental spectral signatures occur in the mid-infrared region (MIR). The overtones and combinational bands can extend to near-infrared or visible spectrum regions. A compilation of these spectral signatures and relevant properties can be found in Türker-Kaya and Huck (2017).

15.2   Common Types of Remote-Sensing Tools Used in Plant Phenotyping

Cameras/sensors with different illumination ranges and capture capabilities (red-green-blue, multispectral, hyperspectral) have been routinely used in diverse studies.

15.2.1   *Visible Light Sensor*

This is a cheap and ubiquitously available sensor. The output (in its simplest form) is an 8 bits per pixel grayscale image. An RGB color image has 24 bits per pixel (one byte for each primary color, red, blue, and green). The range of human eye color perception is quite large, and unsurprisingly, the RGB space does not span the human color gamut. A filter attenuates (or entirely blocks) a subset of wavelengths and allows the complementary subset without attenuation. Many pre-processing options at the sensor and post-processing procedures on the image are available (Pereira et al. 2017; Ngugi et al. 2021).

15.2.2   *Infrared Sensor*

The leaf typically reflects 40–60% of incident near-IR energy from its spongy mesophyll. In terms of data capture/storing data, the IR is identical to a standard camera (visible light). The difference is in the light–object interaction. For example, an IR spectrum can replace commonly used laboratory methods for moisture content measurements like using a gravimetric oven or performing Karl Fischer titration.

15.2.3   *Hyperspectral Sensor*

While visible/infra-red imaging uses a wide range of frequencies (broadband) in the illuminating light source, the hyperspectral imaging sensor generates a series of images, each one representing the intensity distribution within a specific narrow-spectral band. For example, let us consider an object observed through the visible-IR wavelength range between 550 and 850 nm in steps of 5 nm. This set of 61 images, one for each frequency band, is arranged to form a three-dimensional (3D) hypercube used for further processing. This grouping of the frequency data

in each pixel region using narrow bands (hyperspectral imaging) generates a number of independent measurements, thus strengthening any classification/detection procedures (Huang et al. 2014; Schmilovitch et al. 2014).

The most common types of remote-sensing cameras (RGB, multispectral, hyperspectral, light detection and ranging, LiDAR, and thermal) used in agricultural research are unmanned aerial vehicles (UAVs) based or handled spectroradiometers.

- RGB (red, green, and blue) cameras are cheap, most widely used across the platform, and have a high spatial resolution.
- Multispectral cameras: this platform consists of sensors with different lenses. Typically, there will be five sensors (red, green, blue, red-edge, and near-infrared), with each sensor sensitive in one spectral region.
- Hyperspectral cameras cover the 400–1000 nm spectral region with relatively narrow bands (<20 nm).
- Spectrometers cover the 350–2500 nm spectral region with high spectral resolution (1 nm).

The difference between *hyperspectral* and *multispectral* imaging is in their spectral resolution. A multispectral image is a collection of independent data from non-overlapping spectral bands. Usually, the number of bands is few (up to ten). A list of different sensors and their application has been complied in the Index Database (https://www.indexdatabase.de/; Roitsch et al. 2019). A spectrometer (or spectroradiometer) is used to measure both the wavelength and amplitude of the light reflected from the leaf's surface. Wavelength is detected by the position on the sensor that the signal hits. The detected light intensity for each frequency band is obtained using refraction (prism) or diffraction (grating). On the other hand, hand-held spectrometers (Fig. 15.2a) are relatively low cost, easy to collect data and process compared to UAVs.

The key advantage of using hand-held spectrometers is (a) free from confounding effects of illumination, water band, and leaf or camera angle, (b) data processing and analysis require less time than data collected using the UAVs, and (c) data can easily be collected from controlled and field experiments. With the growing interest in sensors application in agriculture, several studies demonstrated the use of hyperspectral data collected from the spectrometer. The spectral bands are often used to establish an empirical relationship between given leaf properties and spectral data. Genomic techniques have been significantly advanced; however, throughput phenotyping remains the major bottleneck to molecular breeding. To take advantage of genomic resources to dissect complex stress tolerance, hyperspectral spectrometers could be used for high throughput phenotyping (Grzybowski et al. 2021). The following sections will provide information on how we can use spectrometers to take advantage of hyperspectral reflectance in plant stress physiology or phenotyping.
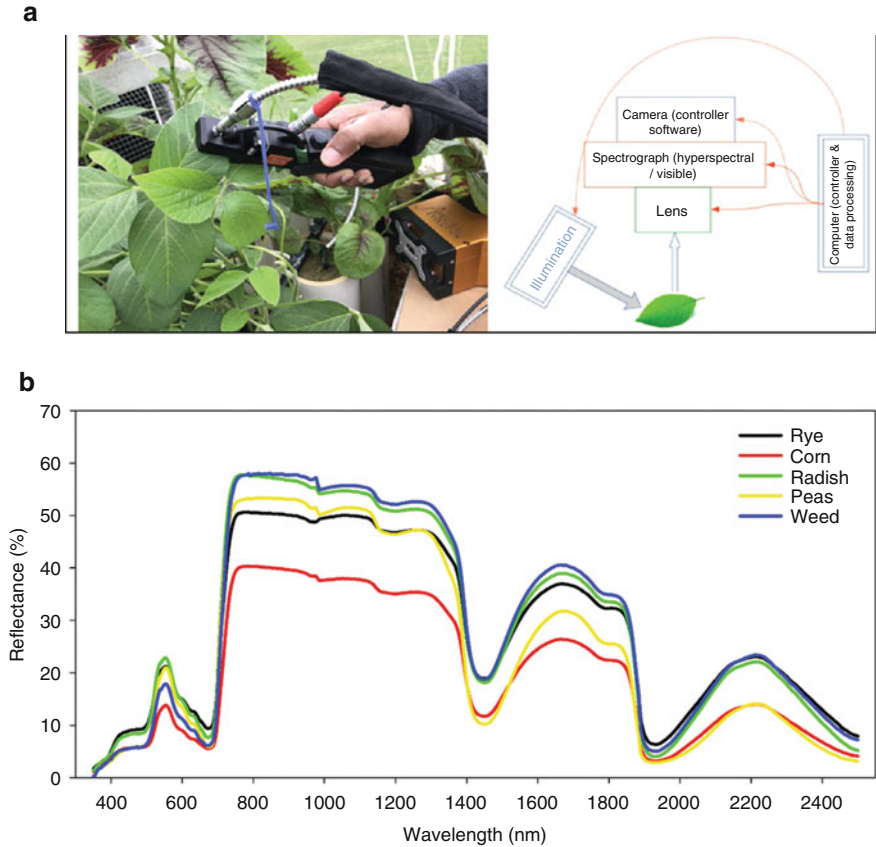
a

b

Fig. 15.2 Handheld spectroradiometer (**a**) and hyperspectral reflectance data (**b**) of different crop species

15.3 Leaf Reflectance Relationship with Plant Phenotypes in Different Crops

A typical leaf hyperspectral reflectance (350–2500 nm) spectrum is shown in Fig. 15.2b. It has low reflectance in the visible region (350–700 nm). Most of the energy is absorbed by the pigments such as chlorophyll and carotenoids for photosynthesis (Zur et al. 2000). There is a peak in the green region (~550 nm, Fig. 15.2b), which has comparatively less absorbance than other colors in the spectrum, causing the leaf to be perceived as the color "green" to the human eye. With changes in leaf pigments due to senescence or species differences, these spectra can show different peaks in the visible region (Sims and Gamon 2002; Morley et al. 2020), showing different colors to the human eye. A comparison of RGB and visible light reflectance implies the possibility of using both to classify plant species (Fig. 15.2b). The first part of the NIR region (700–1200 nm) is dominated by the light scattering at air-cell

wall interfaces and characterized by high reflectance and transmittance. An increase in leaf reflectance at the transition from red to NIR, producing a spectral feature called *red edge* (Gitelson et al. 1996). Researchers have observed correlations between the red-edge position and chlorophyll content, plant stress, plant phenological stages (Yendrek et al. 2017; Silva-Perez et al. 2018; Mir et al. 2019; Reynolds et al. 2020). The second part of the NIR region (1200–2500 nm) is predominantly the light absorption by water. Two leading water absorption bands are at 1450 and 1950 nm (Hoffer and Johannsen 1969), shown in Fig. 15.2b. Leaf absorbance (reflectance) of green light is far less (more) than red light when the plants are stressed.

Observed variations in spectral signatures among the species (Fig. 15.2b) depend on the genetic, physiological, and biochemical properties of the leaf such as temperature, ion-concentration, pigments, concentrations of organic acids, and so on. Chlorophyll pigments reflect strongly around the green band with a green/green-yellow color. Anthocyanin pigments, red in color, provide a high reflectance around the red band. Sugars have a higher reflectance at the NIR. An obvious indication of the temporal change in absorption/reflectance characteristics of visible light on a leaf is color change in response to species or stress or age and nutrient deficiency. Thus, biologists have been perfecting relationships between individual ions and plant health.

15.3.1 Nitrogen and Pigments Associated Leaf Reflectance Spectra

Plants accumulate specific pigments during the courses of growth and development under different stresses. The pigments and their ratios determine the physiological characteristics and plant health status. Chlorophylls are the dominant pigment of green leaves and an expensive molecule because each ring contains four nitrogen atoms. Chlorophyll molecules absorb sunlight at different wavelengths (chlorophyll-a absorbs red-orange light and chlorophyll-b absorbs blue-purple light). As a result, chlorophyll index has been used as a proxy indicator of leaf or canopy nitrogen content and plant health. Traditional methods of determining pigments and tissue nitrogen are costly and time consuming.

Current advances in remote sensing have allowed timely data collection to assess plants' crop growth and nutrient (nitrogen) status. Studies highlighted in Table 15.1 used traditional and sensor-based approaches and identified spectral bands associated with pigments and nitrogen content. Studies reported in Table 15.1 used leaf spectral reflectance data collected using a portable ASD Field Spec FR spectroradiometer (Analytical Spectral Devices Inc., Boulder, CO, USA) compared with the wet lab measured observations (shoot or leaf nitrogen). These studies tested the relationship between the measured values and spectral formulations or indices (Table 15.1). Leaf nitrogen and chlorophyll concentrations of sorghum leaves were

Table 15.1 Summary of selected research papers used leaf reflectance-based vegetative indices or bands in different crops. R represents reflectance at a specific wavelength. R^2 indicates the association between measured and vegetative indices or bands.

Crop	Phenotype	Formula	R^2	Reference
Bermuda grass	Forage nitrogen	R915/R515	0.44	Zhao et al. (2005b)
		R915/R705	0.51	
Castor bean	Carotenoid	R575/R675	0.80	Reddy and Matcha (2010)
		R705/675	0.80	
	Leaf chlorophyll	R545	0.72	
		R555/R675	0.91	
		R555/R455	0.93	
		R705/R655	0.92	
Cotton	Leaf area index	1.16*R800 − R670/ (R800 + R670) + 0.16	0.75	Zhao et al. (2007)
		(R900 − R680)/(R900 + R680)	0.73	
	Leaf carotenoids	R415/R685	0.79	Tarpley et al. (2000)
	Leaf chlorophyll	R415/R695	0.72	
	Leaf nitrogen	R415/R710	0.70	
		R915/R515	0.65	Zhao et al. (2005b)
	Leaf or shoot nitrogen	R915/R705	0.78	
		R715/R405	0.65	Read et al. (2002)
		R795/R755	0.70	
Maize	Carotenoids	R712/R809	0.50	Zhao et al. (2003)
	Chlorophyll a	R712/R1088	0.55	
	Chlorophyll b	R712/R1097	0.58	
	Total chlorophyll	R712/R1088	0.59	
	Leaf nitrogen	R712/R1040	0.55	
		R575/R526	0.69	
Sorghum	Leaf chlorophyll	R555/R465	0.65	Zhao et al. (2005a)
		R555/685	0.62	
		R715/R455	0.62	
		R1075/R735	0.66	
	Leaf nitrogen	R405/R555	0.63	
		R405/R715	0.68	
		R555/425	0.62	
		R715/R415	0.65	
		R1075/R555	0.55	
		R1075/R725	0.65	

significantly associated with the reflectance (R) ratios of R405/R715 ($R^2 = 0.68$) and R1075/R735 ($R^2 = 0.66$) (Zhao et al. 2005a).

Likewise, measured leaf chlorophyll or carotenoids showed a stronger relationship with the reflectance ratios of *R575/R526* ($R^2 = 0.69$) or *R712/R809* ($R^2 = 0.50$) in maize (*Zea mays*), respectively (Zhao et al. 2003). In cotton (*Gossypium*

hirsutum), *R715/R405*, *R795/R755*, *R415/R710*, *R915/R515,* and *R915/R705* showed a stronger association with measured leaf or shoot nitrogen in cotton (Table 15.1). In general, spectral bands *R405*, *R415*, *R515*, *R705*, *R710*, *R755,* and *R915* are associated with leaf nitrogen content or concentration across cotton studies (Tarpley et al. 2000; Read et al. 2002; Zhao et al. 2005b). Further, reflectance ratios of *R555/R455* and *R705/R675* showed strong correlations with total chlorophyll (R^2 = 0.93) and carotenoids (R^2 = 0.80) in castor bean (*Ricinus communis* L.), respectively (Reddy and Matcha 2010). The weakest relation was noted in Bermuda grass between forage nitrogen and *R915/R515* (R^2 = 0.44). The nitrogen and pigment-specific bands (Table 15.1) could be used for non-destructive phenotyping. This information can be utilized to make appropriate nitrogen application decisions in the field.

15.3.2 Correlations of Mid-Day Leaf Water Potential with Spectral Indices

Drought is one of the most significant abiotic stress factors limiting the production of crops. Most field crops are sensitive to drought stress or soil moisture deficit during growth and development (Galieni et al. 2021). High throughput detection or quantification of plant responses to drought stress is one of the most critical crop improvement programs. Here we discuss a study that utilized reflectance indices such as normalized difference vegetation index (NDVI), normalized water index (NWI) in response to evapotranspiration-based irrigation treatments (100, 80, 60, 40, and 20% ET) in soybean. Mid-day leaf water potential was measured 42–57 days after sowing (DAS) using a pressure chamber method (Wijewardana et al. 2019). On the same plants, leaf reflectance measurements were taken between 1100 and 1200 h using a portable ASD spectroradiometer. Soil moisture stress increased soybean canopy reflectance in the visible spectrum range (400–700 nm), especially for 60, 40, and 20% ET compared to the control and 80% ET (Fig. 15.3a). The lower reflectance in the NIR region further confirmed the soil moisture stress-induced reduction in transpiration and stomatal conductance. Beyond the NIR region (1300–2500 nm), which is a function of leaf-water content and leaf thickness, reported to increase spectral reflectance in soil moisture stressed plants. The midday leaf water potential was strongly correlated (Fig. 15.3a) with NDVI (R^2 = 0.82) and NWI16 (R^2 = 0.72). Soil moisture stress-induced reduction in chlorophyll and canopies size resulted in lower NDVI values. These findings suggest that the NIR region could be used as a proxy to describe the canopy mass under water-deficit conditions (Lobos et al. 2014; Elsayed et al. 2015).
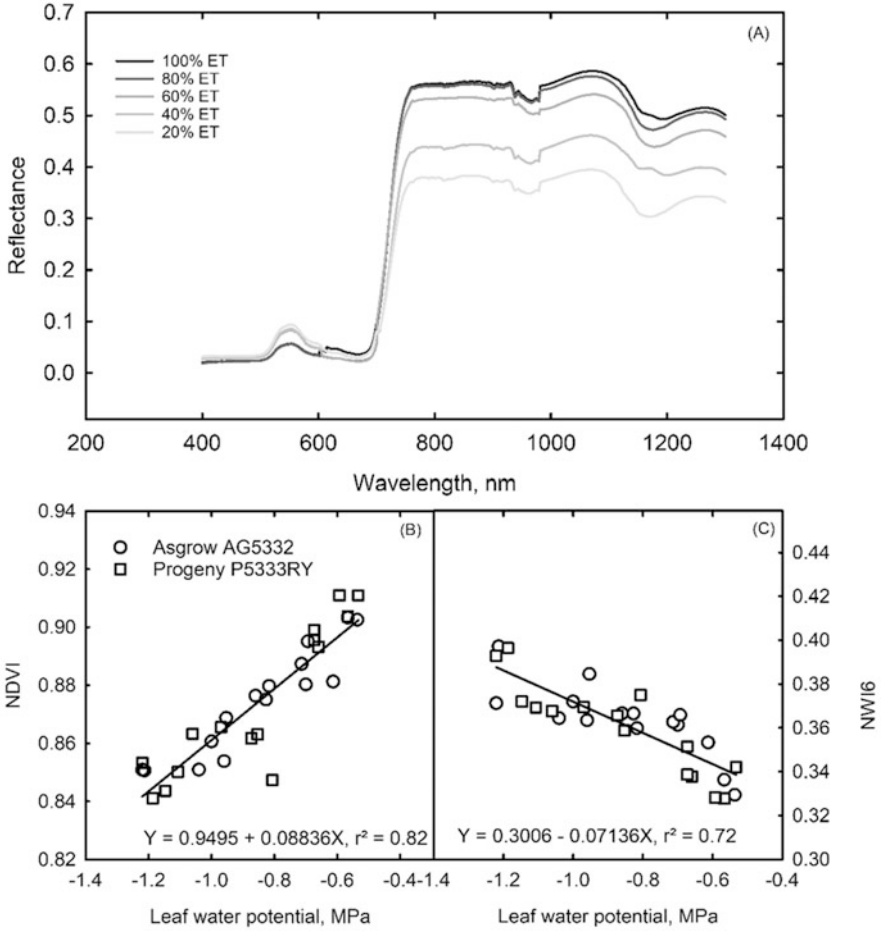
Fig. 15.3 Soybean leaf hyperspectral reflectance of the five soil moisture stress treatments (**a**). The relationships between leaf water potential and leaf spectral indices (Normalized Difference Vegetation Index-NDVI, **b**; Normalized Water Index-NWI, **c**) for soybean subjected to five evapotranspiration-based irrigation treatments. For experimental details refer Wijewardana et al. (2019)

15.4   Advancement in the Analysis of Hyperspectral Reflectance Data

Conventionally, the spectral signatures are used to calculate simple indices, which are then used for testing the correlations among indices and plant phenotype (Table 15.1 and Fig. 15.3). However, this does not necessarily work for all the properties of interest or yield (Grzybowski et al. 2021). Estimating such leaf properties is not a simple regression task since overtones and combinational bands

often contaminate these signatures, especially in the near-infrared (NIR) region. Hence, the leaf spectrum is a complex graph consisting of many overlapping peaks requiring complex multivariate modeling techniques for property estimations (Manley 2014).

The independent information from each band of the hyperspectral image increases the data dimension. While using the full spectral information in a classifier, we can only use a finite number of training samples to estimate many parameters necessary to describe the large data dimension. When the cardinality of the training samples is less than the number of parameters, the estimator performance naturally declines (Dalponte et al. 2009); this effect is called the *Hughes phenomenon*. An optimal pair of training samples, data dimensions exist in most practical circumstances. A dimensionality reduction step (Principal Component Analysis, PCA; Fisher's Linear Discriminant Analysis, LDA; and Stepwise LDA, SLDA) would help bring down redundant features to reduce the dimensions of the hyperspectral observations (Duda et al. 2021). In brief, PCA obtains the best projections of the data. Only a few of the projected components have significant variance (information), and only these fewer "principal" components are used. While PCA is unsupervised and not classification-centered, the LDA (can also be run in supervised mode) maximizes the class separability between the two classes. In SLDA, the discrimination model is refined at each step where a decision on whether to retain or discard a subset of features is taken.

The efficacy of the maximum likelihood (ML) statistical classifier in combination with PCA/LDA/SLDA has been used to classify hyperspectral signatures (Samiappan et al. 2021). These analyses have the capabilities to investigate temporal misalignments between training and testing conditions. These modeling techniques can range from classical partial least squares regression (PLSR) to more modern machine learning techniques such as artificial neural networks, convolutional neural networks, and deep learning (Grzybowski et al. 2021).

15.4.1 Leaf Reflectance for Early Season Disease Diagnosis

Disease diagnosis and accurate estimation of disease incidence are fundamental in agriculture production. For example, the root-knot nematode (RKN) (*Meloidogyne incognita*) is a significant threat to cotton production. The RKN, a soilborne roundworm, threatens cotton and other crops. The formation of RKN affects a series of physiological traits, root system architecture, and final yield. Early diagnosis of RKN is meaningful in reducing disease spread, financial loss and facilitating real-time management practice. Leaf hyperspectral reflectance (350–2500 nm) has been used to understand early-season RKN damages on cotton (Fig. 15.4). Cotton genotypes (Rk-Rn-1, nematode-resistant and M8, nematode susceptible) with contrasting responses to nematode infection at the early vegetative stage were inoculated with nematodes. Temporal hyperspectral reflectance scans were taken 10, 30, and 60 days after the dose of nematode infestation (DAI) using PSR+ 3500 Spectroradiometer
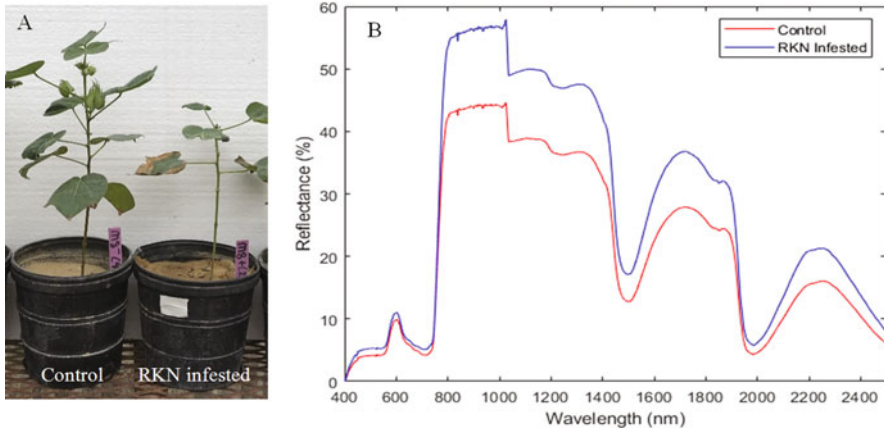
Fig. 15.4 Cotton plant with and without root nematode infestation (**a**). The right graph (**b**) shows the reflectance of control and root-knot nematode-infested leaf of cotton

(Spectral Evolution, MD, USA) to discriminate the cotton plants with invisible RKN disease symptoms. We applied statistical supervised learning algorithms to classify RKN infested cotton from the control group using the spectral range is 350–2500 nm (2150 bands) with a spectral resolution of 2.8 nm at 700 nm, 8 nm at 1500 nm, and 6 nm at 2100 nm full width at half maximum (Fig. 15.4b). A maximum likelihood classifier with a dimensionality reducer revealed temporal hyperspectral changes in response to RKN before the plant's visual symptoms appear. The classifier performed with >90% accuracy using hyperspectral data while classifying healthy cotton plants from RKN infected plants (Fig. 15.4). These results show the possibility of using leaf reflectance data to diagnose RKN infestation in cotton, and the same techniques could be used for other diseases in other crops. The effect caused by the diseases can be non-invasively analyzed using hyperspectral data at the early growth stage.

15.4.2 Crop Species Discrimination Using Hyperspectral Data

Leaf reflectance has been used for monitoring plant health, nutrient status, and stress tolerance in a range of crops (Yendrek et al. 2017; Silva-Perez et al. 2018; Mir et al. 2019; Reynolds et al. 2020; Grzybowski et al. 2021). Differences in leaf morph-physiological properties among different species lead to varying spectral signatures, which can be used for the classification of species (Fig. 15.2), abiotic (i.e., soil moisture stress treatments, Fig. 15.3) and biotic stresses (root-knot nematode, Fig. 15.4). For example, leaf spectral reflectance of showing a clear discrimination between corn and soybean in spectral-domain (Fig. 15.5a) and principal component
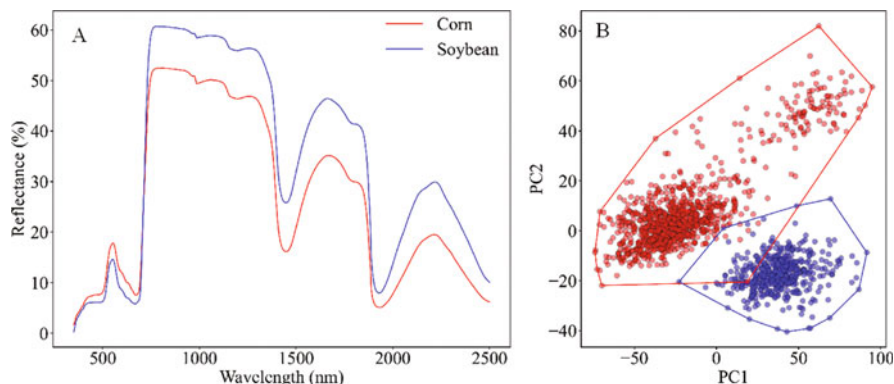
Fig. 15.5 Average corn and soybean leave spectra (**a**) and their separation in principal components (PCs) space (**b**). Corn and soybean leaf hyperspectral signatures were collected from plants grown under controlled environment conditions during the reproductive stage (Bheemanahalli et al., Plant Stress Physiology Lab, Mississippi State University, unpublished). Leaf reflectance observations were collected using the PSR+ 3500 Spectroradiometer (Spectral Evolution, MD, USA)

domain (Fig. 15.5b). It is evident from the results that the two species: corn and soybean can be discriminated utilizing the leaf hyperspectral information (Fig. 15.5). However, this is not always guaranteed, especially when intraspecies spectral variability exceeds interspecies. Natural processes such as leaf senescence, nutrients, and stress can further complicate species separation (Reynolds et al. 2020).

15.4.3 Leaf Reflectance and Soil Nutrients

Spectroscopy has been used in soil analysis for a few decades to estimate soil properties rapidly and cost-effectively. However, uncertainties are associated with estimated soil nutrient content as plant-available nutrients (Singh et al. 2019). The leaf level spectral measurements can bypass the soil nutrient analysis and show the direct impact of available nutrients on plants. Nutrients and minerals in plants in organic and inorganic fractions create spectral signatures used for estimations. For example, leaf N, mainly present in organic form in chlorophyll, absorbs light in the visible region due to the C–C and C=C bonds in the porphyrin rings and the magnesium ion (Sims and Gamon 2002). In addition, the mid-infrared (MIR) region has primary absorptions of many leaf properties such as cellulose, hemicellulose, lignin, xylan, cutin, carbohydrates, and proteins (Türker-Kaya and Huck 2017). Inorganic leaf nutrients such as Ca, K, and other micronutrients can also be derived from spectra due to their association with the organic compound functional groups or organic matrix (Yarce and Rojas 2012; Prananto et al. 2021). A compilation of past studies using near-infrared (NIR) to derive plant nutrients can be found in Prananto et al. (2020). In addition to NIR, the MIR region has also been utilized to derive

numerous plant properties, including cell wall compounds (Jiang et al. 2009; Largo-Gosens et al. 2014), protein structure (Kumar et al. 2016), tissue and taxa differentiation (Huck-Pezzei et al. 2012), and water content (Ullah et al. 2012). Since the leaf level spectral measurements can better indicate the available nutrients in the soil; it can effectively be used in nutrient deficiency diagnosis or management. A study showed that leaf spectra could identify corn's N, P, K, and Ca deficiencies (Al-Abbas et al. 1972). Similarly, other nutrients and physiological processes such as leaf senescence (Ivanova and Singh 2003) can be detected using the leaf-level spectra.

15.5   Future Perspectives

Plant physiologists, geneticists, and breeders are interested in leaf biochemical and physiological properties, usually measured destructively. Destructive measurements limit the speed of phenotyping, where hundreds of samples are needed to characterize variations in nutrients, metabolites, and photosynthetic traits (Grzybowski et al. 2021). Genomic techniques have been significantly advanced; however, throughput phenotyping has remained the primary bottleneck. To take advantage of genomic resources to dissect complex stress tolerance, leaf hyperspectral combined with spectroscopy can serve as an effective and efficient tool for a comprehensive assessment of complex plant performance to stresses. In recent years considerable progress has been made in building spectral features for crop improvement and the technologies to deliver from hand-held instrumentation to drones-assisted platforms. Numerous studies have shown that leaf and canopy spectra can help derive proxies associated with physiological and biochemical traits, which is tremendously helpful in studying heritability, genetic loci discovery, and improving breeding efficiency. Nevertheless, spectral-assisted breeding and selection will gradually evolve as data analysis and tools become more accessible for breeders and physiologists. In the future, spectral-assisted breeding could be seen as a revolutionary approach to enhance genetic gain under various environments.

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