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Close-range hyperspectral imaging of whole plants for digital phenotyping: Recent applications and illumination correction approaches

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ABSTRACT

Digital plant phenotyping is emerging as a key research domain at the interface of information technology and plant science. Digital phenotyping aims to deploy high-end non-destructive sensing techniques and information technology infrastructures to automate the extraction of both structural and physiological traits from plants under phenotyping experiments. One of the promising sensor technologies for plant phenotyping is hyperspectral imaging (HSI). The main benefit of utilising HSI compared to other imaging techniques is the possibility to extract simultaneously structural and physiological information on plants. The use of HSI for analysis of parts of plants, e.g. plucked leaves, has already been demonstrated. However, there are several significant challenges associated with the use of HSI for extraction of information from a whole plant, and hence this is an active area of research. These challenges are related to data processing after image acquisition. The hyperspectral data acquired of a plant suffers from variations in illumination owing to light scattering, shadowing of plant parts, multiple scattering and a complex combination of scattering and shadowing. The extent of these effects depends on the type of plants and their complex geometry. A range of approaches has been introduced to deal with these effects, however, no concrete approach is yet ready. In this article, we provide a comprehensive review of recent studies of close-range HSI of whole plants. Several studies have used HSI for plant analysis but were limited to imaging of leaves, which is considerably more straightforward than imaging of the whole plant, and thus do not relate to digital phenotyping. In this article, we discuss and compare the approaches used to deal with the effects of variation in illumination, which are an issue for imaging of whole plants. Furthermore, future possibilities to deal with these effects are also highlighted.

1. Introduction

1.1. Plant phenotyping

Plant phenotyping is the measurement of the interaction of a plant with its surrounding environment (Costa et al., 2019; Pieruschka and Schurr, 2019). Phenotyping related experiments are widely performed in the plant science domain and in breeding programs. These experiments assess the performance of plants when they are exposed to different environmental conditions and enable the identification of the best performing genotypes, which can be taken forward and used in the development of high-quality plants (Zhao et al., 2019). The quality factors are based on the improvement of some unique traits such as drought tolerance, salt-resistance, post-harvest quality of fruits, increase in the number of seeds, fruit loads and many more (Fiorani and Schurr, 2013). Selection of the best-performing plants and breeding has been performed since the start of agriculture by humans (Diamond and Ordunio, 1999). However, it was limited by the experience of farmers and was not based on any scientific evidence. Advancement in science and technology has given rise to a detailed understanding of plants. The behaviour of plants can be monitored during breeding experiments and phenotyping in a controlled optimized high-throughput way (Furbank and Tester, 2011; Bucksch et al., 2014; Li et al., 2014; Walter et al., 2015; Roitsch et al., 2019). Nowadays, high-throughput breeding experiments are performed worldwide in both public research as well as industrial facilities (Fahlgren et al., 2015; Lee et al., 2018; Rouphael

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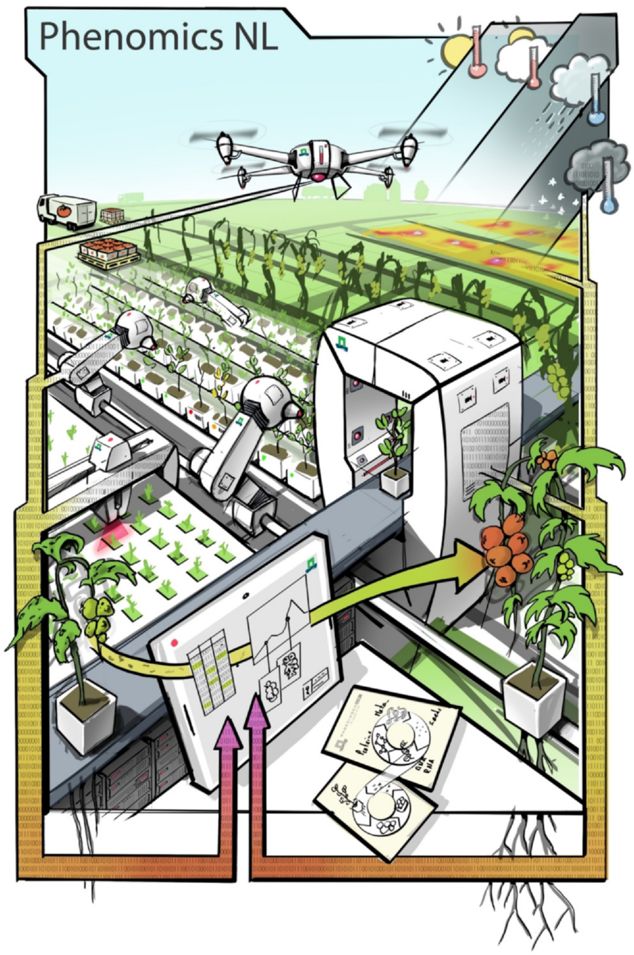

Fig. 1. A scenario of complete digital phenotyping from lab to field. (Image courtesy and reuse permission granted by: Rick van de Zedde, Wageningen University & Research, The Netherlands). The figure is inspired from the vision of Netherlands Plant Eco-phenotyping Centre (NPEC).

et al., 2018; Yang et al., 2020). A summarised example of the digital phenotyping form lab to the field is presented in Fig. 1. Digital phenotyping comprises of three main parts: automation and robotics, non-destructive sensing, and advanced data modelling and analysis. All these components synergistically follow and models plant features along its growth under phenotyping experiments.

Two main types of features (physical and chemical) can be followed for an above ground plant during a phenotyping experiment (Mishra et al., 2017). Physical features include structural changes in plants such as the development of leaves, increase in height, number of leaves, leaf area etc. Chemical features include changes in pigment composition, moisture content, proteins, carbohydrates, sugars and several other plant related chemicals. The structural features and chemical composition depend on the type of plant under study and the environment under which it is grown (Dhondt et al., 2013; Yang et al., 2020). The measurement of structural traits is easier when compared with chemical composition, as structural traits are observable to humans (Li et al., 2014). However, humans cannot follow enormous breeding experiments that require the monitoring of hundreds of plants subjected to multiple treatments over several months. Until the last decade, plant phenotyping was marked as a time-consuming and labour-intensive task. However, the emergence of high-throughput phenotyping platforms (HTPPs) has enabled the automation of phenotyping experiments (Yang et al., 2020). Imaging technology has played a major role in automating the monitoring and tracking of the structural changes in plants during the phenotyping experiments (Dhondt et al., 2013; Li et al., 2014; Mishra et al., 2017). This has been accompanied with advances in data processing algorithms to perform tasks such as plant segmentation and 3D reconstruction of plant shape to extract information such as leaf structure, number of leaves, leaf area and other physical characteristics (Li et al., 2014; Lobet, 2017; Tardieu et al., 2017; Rahaman et al., 2019). However, extraction of the chemical properties of plants through non-destructive methods is still a major challenge. Typically, chemical analysis requires destructive sampling and involves highly sophisticated laboratory-based experiments. This is not desirable in digital phenotyping as destructive sampling prevents monitoring of the plant over time.

1.2. Hyperspectral imaging for digital phenotyping

A hyperspectral camera contains a spectrograph. The spectrograph is used to split incoming spectra into discrete wavelengths. The output of a spectrograph is recorded on a detector. A powerful and stable light source is required for the acquisition of hyperspectral data. HSI can be performed in multiple modes such as push broom line scan, point scan and snapshot. Push-broom line scan cameras are mostly used due to their speed advantage over point scan cameras, **high-quality data** and market readiness compared to snapshot cameras. Specific details on different types of HSI systems can be found in a published technical review on HSI (Qin, 2010).

The main benefit of utilizing HSI compared to other imaging techniques is the ability to extract structural and physiological information simultaneously from plants (Lowe et al., 2017; Mishra et al., 2017). The structural information (physical structure of plant such as leaves and stems) is readily available from the imaging modality of HSI whereas the physiological information can be retrieved from the spectral information present in each pixel of the imaging scene. In the spectral range of 400–700 nm, the composition of plant pigments such as chlorophyll, anthocyanin, carotenoid can be extracted. In the spectral range 700–2500 nm, information related to compounds containing C-H, N-H and O-H bonds can be extracted (Mishra et al., 2017) (Jacquemoud and Baret, 1990; Jacquemoud and Ustin, 2019). Together, the structural and physiological information provides enhanced understanding of the functional dynamics of plants. Further, since HSI is a non-destructive technique, it enables the tracking of plants over the complete course of the phenotyping related experiment. The recent focus on digital phenotyping has led to the integration of HSI in HTTPs as a continuous plant monitoring tool to support phenotyping experiments under controlled greenhouse conditions (Asaari et al., 2018; Asaari et al., 2019; Mishra et al., 2019b; Mishra et al., 2020a,b). HSI is typically integrated in HTTPs within a closed room with standard illumination conditions, i.e., using a halogen lamp to cover the near-infrared spectral region (Asaari et al., 2018; Asaari et al., 2019). During an experiment, the plant is brought to the closed room with a conveyor system and image acquisition is performed (Asaari et al., 2018; Mishra et al., 2019b). For phenotyping in the field, HSI is implemented in robotic field scouting platforms to perform imaging of crop lines, and there is increasing integration of HSI in drones to enable aerial imaging (Polder et al., 2019; Mishra et al., 2020a,b).

Digital phenotyping is emerging as a key research domain at the interface of agriculture, technology and plant science (Awada et al., 2018; Liu et al., 2019). The key aim of digital phenotyping is to deploy high-end non-destructive sensing techniques and information technology infrastructure to automate the extraction of both structural and physiological traits. A widely used technique in the digital phenotyping domain is hyperspectral imaging (HSI) (Mishra et al., 2017). The use of HSI was mostly limited to remote sensing applications. However, close-range HSI has emerged as a potential tool for *in-situ* non-destructive rapid assessment of plants in recent years (Li et al., 2014; Lowe et al., 2017; Mishra et al., 2017).

When electromagnetic radiation (EMR) strikes the surface of a plant, it can be reflected, transmitted and absorbed (Mishra et al., 2017). The extent of reflection after the interaction of EMR and the plant, depends on the physicochemical attributes of the plant (Mishra et al., 2020a,b). These attributes range from the plant chemical composition such as the moisture, sugar, pigments, protein and other plant specific biochemical properties, as well as the physical properties of the leaves (Jacquemoud and Baret, 1990; Mishra et al., 2017). The reflected spectrum from a plant over a specific wavelength range is termed the spectral signature or if the average reflectance is noted then it is called the reflectance of the plant. Devices like spectrophotometers and hyperspectral cameras are used to obtain these spectral signatures. A spectrophotometer can provide spectral information about a small area at a selected location on a plant. The spectral resolution of spectrophotometers is high but they do not provide any spatial information. A hyperspectral camera can acquire high resolution data in terms of both the spatial and spectral properties of an object.

1.3. Challenges associated with close-range hyperspectral imaging of plants

The use of HSI for the acquisition of plant images is a challenging task owing to the complicated imaging setup. These challenges include selection of a spectral sensor, covering the appropriate wavelength range, selection of an illumination source, and integration of the HSI setup with existing HTPPs (Mishra et al., 2017). Further, there are also factors to consider such as heating of the plant inside the imaging cabinet, which gives extra stress to the plants and introduces bias to the phenotyping experiments. Most of these challenges are minor and have already been dealt with by technical modifications. For example, the spectral sensor and wavelength range can be selected based on the plant traits of interest and the heating of plants can be controlled by deployment of air-conditioning in the imaging cabinet. The interaction of light with the complex geometry of plants gives rise to light scattering, shadowing, multiple reflections and a complex mixture of all these effects depending on the plant (Mishra et al., 2017). This causes variations in illumination, which masks the actual spectral responses from plants and hence, affects the data modelling task. Currently, no solution has been developed to fully correct for variations in illumination in HSI of plants. However, a number of studies have tried to deal with the effects of variations in illumination using spectral normalization (Asaari et al., 2018; Asaari et al., 2019; Mishra et al., 2019a), spectral projections (Al Makdessi et al., 2019), local shape information fusion from 3D sensors (Behmann et al., 2016; Huang et al., 2018) and by modification of the radiative transfer model by the integration of specular reflection at the leaf surface and local leaf inclination (Jay et al., 2016; Morel et al., 2018).

To the best of our knowledge, this is the first study to review and summarize the challenges of close-range HSI of whole plants and the different approaches that are being deployed for correction of variation in illumination. Hence, the present article first provides a basic introduction to the HSI of plants. Further, it presents a comprehensive review of recent studies related to close-range HSI of whole plants. Close range denotes a distance of up to 1–2 m from the plant top. Several studies have used HSI for plant analysis but were limited to imaging of leaves, which is considerably more straightforward than imaging of the whole plant, and thus do not relate to digital phenotyping. Therefore, in the present work the focus is on the use of close-range HSI of whole plants. The approaches used to correct for variations in illumination, and their advantages and disadvantages for the HSI of whole plants are discussed. Furthermore, future possibilities to deal with these effects are discussed.

2. Underlying cause of variation in illumination in close-range hyperspectral imaging

HSI of whole plants is performed in diffuse reflectance mode and from the top view to capture the complete canopy (Fig. 2a). Along with each image, a dark current and white reference (usually Spectralon) are also captured. The dark current and white reference are then used for radiometric calibration of the raw images to estimate the percent reflection from the plant with respect to the standard white reference. However, the white reference does not allow the correction of illumination differences on the surface of plants that are present due to the plant's complex geometry (Mishra et al., 2017). As shown in Fig. 2b, when the light interacts with a flat white reference, it is reflected without any further attenuation except for the interaction with the surface of the white reference. However, when light interacts with plants, several complex phenomena such as multiple reflections, nonlinear mixing of multi-reflections, shadowing of leaves, and the interaction of shadowing and multi-reflections might occur (Mishra et al., 2017; Al Makdessi et al., 2017). The dominance of these phenomena is dependent on the complexity of plant geometry. For example, a plant with a few broad leaves might exhibit less of these illumination effects than a grassy dense plant. The final signal captured by the camera will contain the plant's specific physicochemical information but it will be masked by variations in the signal due to the complex interaction of illumination with plant geometry. Attenuation bought about by variations in illumination can range from simple intensity differences to complex high-order multiplicative and additive effects (Asaari et al., 2018). Any data modelling performed without reducing/removing these illumination effects may lead to suboptimal conclusions.

3. Recent studies on whole plants

Applications of close-range HSI for exploring complete plants are emerging. The present work identified a total of 16 applications as of now (June 2020) where HSI was used to analyse complete plants. There is a low number of applications in the scientific literature as the use of HSI for digital phenotyping is still under consideration. Furthermore, the challenge of variations in illumination still limits the full exploitation of the rich information generated by HSI. A summary of applications is provided in Table 1. The applications considered in this article were from both controlled greenhouse conditions as well as open fields. Based on Table 1, it can be understood that, currently, applications of HSI of complete plants are mainly for 4 purposes: monitoring and detection of biotic and abiotic stress; prediction of biochemical parameters; testing of the potency of crop protection compounds; and detection of disease in plants. Further, the approaches developed and adopted for illumination correction such as spectral averaging (Pandey et al., 2017), spectral normalization (Asaari et al., 2018), deep learning (Polder et al., 2019), oblique projections (Al Makdessi et al., 2019) and fusion of 3D shape and hyperspectral information (Sun et al., 2019) were identified. However, several works were also found where illumination effects were not considered (given in Table 1). There were no studies that compared the use of different illumination correction methods. The data processing approaches used for pattern recognition and predictive analysis ranged from latent variable based chemometric methods such as partial least square regression (PLSR) (Pandey et al., 2017) to high-end convolutional neural network-based deep learning algorithms (Polder et al., 2019). Applications involving HTPPs were found mainly to follow the physicochemical changes in plants arising from abiotic stress such as drought (Asaari et al., 2018; Asaari et al., 2019).

4. Illumination correction approaches

Illumination correction in close-range HSI of plants is the main step prior to information extraction. There is no clear solution to illumination correction but several works (Table 1) were found where variations in illumination were removed and mitigated utilizing diverse approaches. The approaches identified in Table 1 are described in the following section. A summary of key approaches is also provided in Fig. 3.

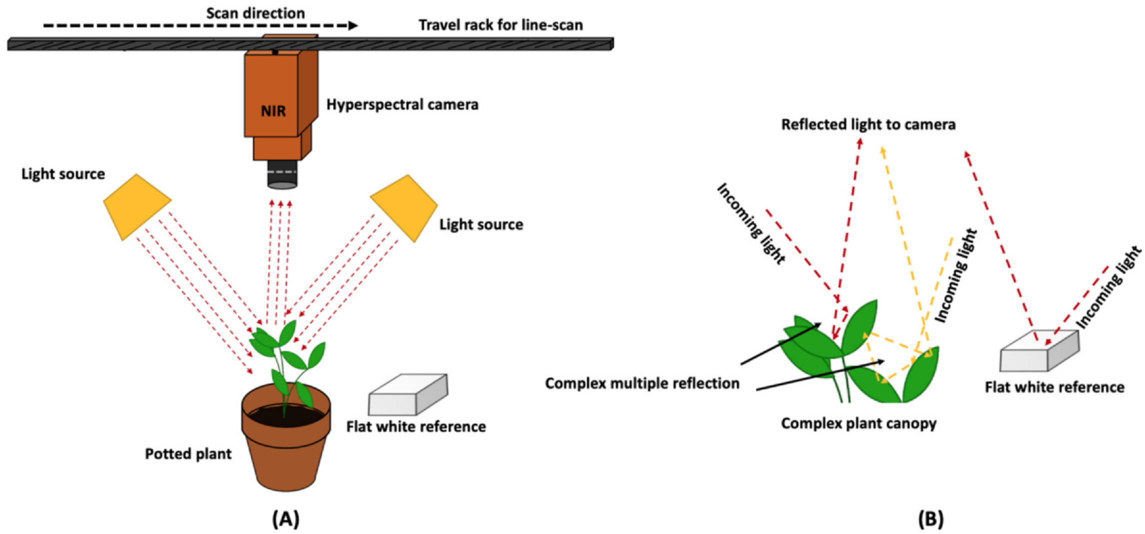

Fig. 2. A schematic of close-range hyperspectral imaging of plants. (A). A potted plant being imaged with a push broom hyperspectral camera mounted on a travel rack, and (B). The complex interaction of light with the plant canopy.

4.1. Spectral averaging and pixel normalization

The most straightforward approach for reducing the effects arising from variations in illumination is spectral averaging. Spectral averaging in HSI refers to calculation of the average spectrum over the pixel domain. However, before averaging, segmentation of the images should be performed. Averaging should involve the pixels from the plant and not from any other background objects. The underlying assumption of averaging is that the effects arising from variation in illumination add noise to spectra. Therefore, averaging many spectra from the same plant can allow the estimation of an averaged response with less noise and so reduced illumination effects. Several recent works used the averaging approach to process HS images and obtained the benefits of using HSI for prediction of biochemistry (Ge et al., 2016; Pandey et al., 2017; Bruning et al., 2019) and drought detection (Mazis et al., 2020; Weksler et al., 2020). However, no work compared spectral averaging with the other approaches reported in the scientific literature (Table 1). The main benefit of averaging is that it does not require extra sensor measurements and is relatively fast. However, there are two main disadvantages of the averaging approach. The first one is that it is highly dependent on the image segmentation quality to extract regions that correspond to only plants in the spectral images. If segmentation is poor, averaging will consider the pixels that are non-plants. The second problem with averaging is the loss of spatial information, which is at odds with the reasons for the use of hyperspectral cameras. In all of the studies (Table 1) that reported the use of averaging, the user discarded the spatial information from HSI. Based on the studies reviewed it is recommended that the user should not use averaging as it leads to a loss of spatial information.

4.2. Spectral pre-processing inspired from chemometrics

The second approach is inspired by the chemometrics domain, where light scattering effects are frequently encountered. In chemometrics and especially for data generated with spectroscopic techniques, it is assumed that the spectral information consists of absorption characteristics. These characteristics are related to the underlying chemical components and the scattering characteristics correspond to the complex interaction of light with the physicochemical properties of the samples (Roger et al., 2020b). The scattering information, however, masks the signal corresponding to the chemical components, thus affecting the data modelling. The presence of scattering effects can be noted as additive and multiplicative. Therefore, in chemometrics, the aim is to utilise scatter correction techniques to remove the effect of scattering from the spectral data and what is left behind is the absorption characteristics (Rinnan et al., 2009; Roger et al., 2020a). Modelling based on the absorption characteristics leads to optimal models. In the HSI of plants, a popular chemometrics spectral normalisation technique called standard normal variate (SNV) has been widely used (Asaari et al., 2018; Asaari et al., 2019; Mishra et al., 2020a,b). SNV pre-processing involves the transformation of a spectrum by subtracting its mean and dividing by its standard deviation (Barnes et al., 1989). By performing this transformation, the spectra affected by scattering are normalised with the subtraction of mean. This allows the correction of global intensity differences and division by the standard deviation corrects for the multiplicative part of the scattering effect (Roger et al., 2020a). However, it should be noted that the SNV transformation is performed individually for each pixel, and the estimation of the mean and standard deviation does not depend on any other neighbouring pixels. This is one of the benefits of SNV over the averaging approach where the presence of a non-plant pixel can affect the averaging operation. Apart from SNV, other chemometrics approaches to scatter correction such as 2nd derivative (Roger et al., 2020a), multiplicative scatter correction (MSC) (Isaksson and Næs, 1988) and extended multiplicative scatter corrections (EMSC) (Martens et al., 2003) were also used (Bruning et al., 2019). However, the main advantage of SNV over the aforementioned methods (MSC and EMSC) is that it does not require any extra reference measurements. SNV also does not require any other extra sensor measurements, unlike methods such as 3D shape and HSI fusion. In some works (Table 1), SNV correction has been used after the averaging operation (Bruning et al., 2019; Weksler et al., 2020). Such use might help to remove the scattering form the average spectra but does not help to regain the spatial information. Therefore, our recommendation is that the user should use the scatter correction approaches directly at the pixel level and not on averaged spectra.

4.3. Fusion of 3D and hyperspectral imaging

Plants have complex geometries as they comprise multiple leaves (different sizes) at different curvature and at different distances from the camera (based on the height of plants). However, with HSI only spatial and spectral information is captured. Shape information can provide a better understanding of plant geometry, and in addition, precise shape information can be used to correct for the effects of variation in illumination due to local inclination even at the leaf level.

Table 1
A summary of recent studies of close-range HSI of complete plants.

Application interest	Spectral range (nm)	Camera model	Illumination source	Environment	Plant species	Illumination correction	Data modelling	References
Early drought stress detection	394–890	SOC-700 (Surface optics, USA)	6 halogen lamps (400 W ECO, OSRAM, Munich, Germany) from a distance of 1.6 m	Rainout shelter and field conditions	Barley (*Hordeum vulgare L.*)	Not mentioned	Simplex volume maximization (SiVM)	(Romer et al., 2012)
Early drought stress detection	430–890	SOC-700 (Surface optics, USA)	6 halogen lamps 400 W each	Greenhouse and field	Barley (*Hordeum vulgare*) and Maize (*Zea mays*)	Not mentioned	Support vector machine (SVM)	(Behmann et al., 2014)
Water content prediction	550–1750	Headwall Extended VNIR (Headwall Photonics, USA)	N.I. but should be halogen lightening	Greenhouse	Maize Plants (*Zea mays*)	Average Spectra	PLSR	(Ge et al., 2016)
Concentrations of macronutrients nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S), and micronutrients sodium (Na), iron (Fe), manganese (Mn), boron (B), copper (Cu), and zinc (Zn)	550–1700	Headwall Extended VNIR (Headwall Photonics, USA)	N.I. but should be halogen lightening	Greenhouse	Maize (*Zea mays*) and soybean (*Glycine* max)	Average Spectra	Partial least square regression (PLSR)	(Pandey et al., 2017)
Early drought stress detection	400–1000	ImSpector V10E (Specim Imaging, Oulu, Finland)	9 (3 × 3) equally spaced 35 W halogen lamps	Greenhouse	Maize plants (*Zea mays*)	Standard normal variate (SNV)	K-means, Spectral angle mapper	(Asaari et al., 2018)
Water and N content	400–1000 and 1300–2500	Fx10 and SWIR (Specim, Oulu, Finland)	18 halogen light	Greenhouse	Wheat (*Triticum aestivum*)	Spectral averaging followed by 1st and 2nd derivatives, multiplicative scatter correction (MSC), Extended multiplicative scatter correction (EMSC), normalized by range, SNV and spectral smoothening	Principal component regression (PCR), PLSR, multi-linear regression (MLR), SVM and random forest	(Bruning et al., 2019)
Drought stress induction and recovery	400–1000	ImSpector V10E (Specim Imaging, Oulu, Finland)	9 equally spaced 35 W halogen lamps	Greenhouse	Maize (*Zea mays*)	SNV	K-means	(Asaari et al., 2019)
Testing crop protection compounds on plants	400–1000	Headwall VNIR (Headwall Photonics, USA)	2 halogen bulbs	Greenhouse	Not mentioned (confidential due to industry publication)	SNV	Principal component analysis (PCA)	(Mishra et al., 2019b)
Early drought stress detection	400–1000	Headwall VNIR (Headwall Photonics, USA)	2 halogen bulbs	Portable setup/ greenhouse	Arabidopsis (*Arabidopsis thaliana*)	SNV	K-means	(Mishra et al., 2019a)
N, P and K content	400–1000	SOC-710 (Surface optics, USA)	150 W halogen bulbs (Number not mentioned)	Greenhouse	Tomato plants (*S.lycopersicum*)	RGB + D image for registering hyperspectral data	Back propagation artificial neural network (BPANN), SVM and Gaussian process regression (GPR) for regression and PCA and random Frog (RF) for variable selection	(Sun et al., 2019)
Water content	400–1000	Gaia hyperspectral imaging	N.I.	Greenhouse	Maize (*Zea mays*)	Not mentioned	Wavelength selection with PCA and Kullback-Leibler (KL) divergence and SVM regression	(Gao et al., 2019)
Potato virus Y detection	400–1000	Fx-10 (Specim, Oulu, Finland)	13 Tungsten Halogen lamps (Osram Decostar 51 PRO, 14 Watt, 10°, Dichroic)	Field	Potato plants	Black box (Deep learning)	Deep learning convolution neural network	(Polder et al., 2019)
Drought	900–1700	NIR-300PGE (Vosskühler GmbH, Germany)	Radium Ralogen PAR16 35 W	Greenhouse	Grapevines (Vitis vinifera L.)	Not mentioned	Not defined	(Briglia et al., 2019)

(*continued on next page*)

Table 1 (*continued*)

Application interest	Spectral range (nm)	Camera model	Illumination source	Environment	Plant species	Illumination correction	Data modelling	References
Leaf nitrogen content	400–1000	HySpex VNIR-1024 (Norsk Elektro Optikk, Norway)	N.I.	Grrenhouse	Wheat (*Triticum aestivum*)	Oblique projections	PLSR	(Al Makdessi et al., 2019)
Early drought detection	545–1700	Headwall Extended VNIR (Headwall Photonics, USA)	Halogen bulbs	Greenhouse	Oak (Quercus bicolor Willd. and Quercus prinoides Willd)	Average spectra	Vegetation indexes (VIs)	(Mazis et al., 2020)
Transpiration rate	400–1000	Fx10 (Specim, Oulu, Finland)	Diffused natural conditions	Greenhouse	Pepper plants (*Capsicum annuum*)	Averaged spectrum followed by SNV	Correlation analysis	(Weksler et al., 2020)

*N.I.: not indicated.

Some recent works realised this and performed a fusion of hyperspectral information with 3D shape information (Behmann et al., 2016; Huang et al., 2018). The main task in 3D shape and hyperspectral data fusion is to relate the two modes to a unique coordinate system. This has been performed by geometrical calibration and a reference system was developed through a reference object (Behmann et al., 2015; Behmann et al., 2016). In this way, the depth information was projected to the hyperspectral image coordinate system and assigned to the single pixels. The final models were generated by subsampling the 3D shape information and performing a nearest neighbour assignment to the hyperspectral data. The results from application of 3D-hyperspectral models to sugar beet plant showed improved detection of disease-related changes (Behmann et al., 2016). In another work, the 3D shape information and the hyperspectral data were registered with the iterative closest point algorithm (Sun et al., 2019). The 3D-hyperspectral models were then used for predicting the nitrogen, potassium and phosphorus contents in tomato plants. The results showed that the predictive performance of a model constructed using 3D-hyperspectral data was more stable compared to those without information fusion (Sun et al., 2019).

Although 3D-hyperspectral models look a promising approach, they still lack wider application. A major challenge with the 3D-hyperspectral model is the requirement for calibration of sensor depth and an image registration step, which leads to extra measurement time per plant. The memory requirements and computation time for data acquisition also bring challenges for this approach. In a high-throughput phenotyping setup, this might be challenging to achieve. On the other hand, averaging and spectral normalisation are computationally inexpensive and do not require any extra measurements or hardware setup. Currently, there are more realistic applications of spectral normalisation and averaging in high-throughput phenotyping compared to 3D-hyperspectral models (Table 1).

4.4. Oblique projections

Oblique projection is a chemometric approach to spectral correction, which allows removal of multi-scattering effects in close-range hyperspectral images of vegetation scenes (Al Makdessi et al., 2019). The oblique projection approach assumes that the spectra from hyperspectral images are made up of two parts, a useful part which is related to the property of interest and a non-useful part arising from scattering and the effects of variation in illuminations. A simple way to remove the non-useful part from data is via orthogonal projection approaches (Roger, 2016). However, the non-useful part has a non-empty intersection with useful information, and so complete removal of the non-useful part can lead to loss of information (Al Makdessi et al., 2019). The oblique projection approach entails a non-orthogonal spectral projection such that information loss is minimal with maximum removal of the effects arising from variations in illumination. The oblique projection also requires definition of the useful and non-useful subspaces before performing any projection. The useful subspace is straightforward to define and is obtained as the latent variables of the PLSR model related to the property of interest. The non-useful subspace, i.e., illumination effects, is defined using the spectra and estimating polynomial terms up to a maximum degree (Al Makdessi et al., 2019). The user can choose the degree of polynomial based on the complexity of the illumination effects. Once both the useful and non-useful spaces are defined, the oblique projection is performed, and the projection matrix is obtained. Finally, the new model is built by transforming the spectra based on the projection matrix. An application of oblique projection for N content prediction in wheat plants showed improved prediction compared to no correction (Al Makdessi et al., 2019). Although this technique does not require any extra sensor measurements, it requires extra simulations to define the non-useful subspace comprising illumination effects. The advantage of oblique projection over the averaging approach is that it retains the spatial

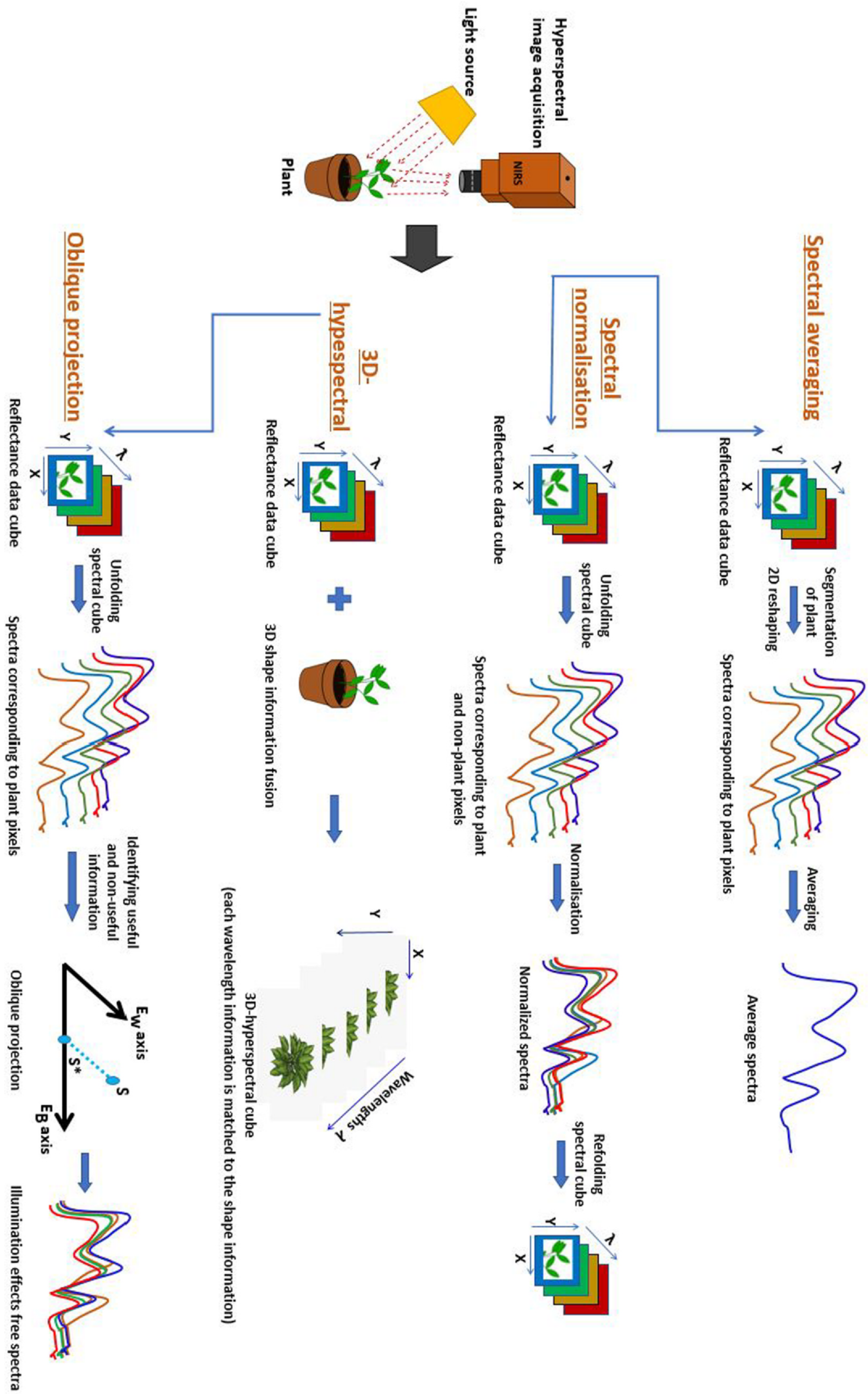

Fig. 3. A summary of key illumination correction approaches.

information in the scene. However, it does not provide any significant advantages over the spectral normalisation method. Further, compared to averaging and spectral normalisation, applications of the oblique projection approach are still lacking for high-throughput scenarios.

4.5. Radiative transfer modelling

Radiative transfer models are physics based models that simulate light propagation within plant leaves/canopies as a function of the leaf biochemical constituents, leaf anatomy and the canopy structure (Jacquemoud and Baret, 1990; Jacquemoud et al., 2009; Jay et al., 2016). Radiative transfer modelling is common in remote sensing, where it is often used for the retrieval of physicochemical properties of plants at the leaf and canopy levels (Jacquemoud and Baret, 1990; Jacquemoud et al., 2009). However, the radiative transfer model does not apply well to close-range hyperspectral imaging since the directional effects are not considered in the available models. A new hyperspectral radiative transfer model for hyperspectral data called PROCOSINE has been developed (Jay et al., 2016). PROCOSINE involves the integration of two extra terms (specular reflection and the local leaf orientation) specific to plant modality to the PROSPECT model. Application of the PROCOSINE model showed that including the specular reflection and the local leaf orientation in the model resulted in a robust estimation of leaf properties. PROCOSINE can be used for the rapid estimation of the spatial distribution maps of physicochemical properties in leaves (Jay et al., 2016). In another leaf scale application, PROCOSINE led to an improved estimation of leaf biochemical and biophysical parameters, which were used to classify different stages of plant disease (Morel et al., 2018). As PROCOSINE considers the local inclination, it provides a rapid estimation of the physicochemical properties just based on the numerical inversion and pseudo-bidirectional reflectance factor hyperspectral measurements (Jay et al., 2016). Currently, the applications of the PROCOSINE approach are limited to only leaf scale. The technical challenges of implementing PROCOSINE for a complete plant is unclear.

4.6. Deep learning

Deep learning is an emerging and promising field in the area of machine learning. Deep learning is inspired by the use of neural networks. These neural networks consist of several hidden layers and are able to extract discriminant information from the input data to perform a given task, for example classification and segmentation etc. Deep learning is an emerging approach to solve applications related to image processing. Deep learning has the benefit that it can model the highly non-linear patterns in the data. The disadvantage of deep learning is the lack of inference for the trained model. Currently, a single application related to deep learning on hyperspectral imaging of plants for disease detecting exists (Polder et al., 2019). The application utilises the convolutional neural networks (CNNs) to classify the diseases in the potato plants. The work showed promising results from data from multiple seasons. However, there is no mention of the illumination effects present in the hyperspectral images of potato plants. It is most likely that deep learning up to a certain extent has modelled the variation caused by illumination effects separate to the useful variation. However, the study does not compare or discuss any illumination correction approach. Deep learning can be a useful tool compared to the 3D hyperspectral model and radiative transfer modelling, but it is limited to the modelling of large datasets. Applications of deep learning for plant phenotyping can be promising when sufficient data is available for training the model.

5. Discussion

In recent years, application of HSI for close-range imaging of complete plants has increased rapidly. In terms of its adoption in digital phenotyping platforms, the present review has highlighted that close-range HSI has been used for four main tasks: early stress detection, testing the potency of crop protection compounds, biochemistry prediction and disease detection. The major challenge of HSI of plants, i.e., correction of the effects of the variation in illumination, is dealt with in multiple ways. Several approaches for illumination correction such as spectral averaging, spectral normalization based on chemometrics, deep learning, oblique projections and fusion of 3D shape and hyperspectral information were identified. However, several works were also found where the illumination effects were not considered. A practical comparison of several illumination correction approaches to decide on the optimal solution is still lacking.

Until now the easiest and most reliable model-free approach to illumination correction seems to be spectral normalization based on chemometrics. Spectral averaging is also a model-free approach, but it removes the rich spatial information present in the hyperspectral scene. The practical applications of 3D hyperspectral modelling and radiative transfer modelling are still lacking from the perspective of digital phenotyping and are currently limited to either individual plant or leaf level. Deep learning seems to work fine but it does not clearly explain the modelled illumination effects, which means that it might be difficult to generalize the correction approach. In some works, spectral normalisation has been used after spectral averaging. Such use might help to remove the effects of variation in illumination from average spectra but it does not help to regain the spatial information. Therefore, the user should use spectral normalisation directly at the pixel level and not on averaged spectra. In most of the cases related to spectral normalisation, a dominance of the SNV approach can be found. SNV works fine but technically, it is not an optimal technique for illumination correction as it assumes that all of the spectral wavelengths are affected in the same way (Roger et al., 2020a). A newly proposed spectral normalisation approach called variable sorting for normalisation (VSN) could be an alternative to SNV (Rabatel et al., 2020). VSN defined the weighting of wavelengths based on the amount that they are affected by variations in illumination. In such a way, VSN simply removes the illumination effects while keeping useful information intact (Mishra et al., 2020a). Recent application of VSN to remove/mitigate the illumination effect showed that it is more efficient than the SNV approach in removing the illumination effects from the HSI data of plant (Mishra et al., 2020a). As an example the results of VSN correction for potato plant under close range HSI are presented in Fig. 4. After the correction with VSN, the intensities light intensity variations (Fig. 4C, E) was normalised to a major extent (Fig. 4D, F). The effect of two different pre-processing on the spectra of HSI are presented in Fig. 5. It can be noticed that prior to any pre-processing the spectra has differences in global intensities of the pixels caused by the illumination differences. The SNV pre-processed spectra was able to avoid the illumination effects but the information was mainly limited to the color differences. The VSN pre-processed spectra highlighted the main differences in the chlorophyll and the red edge parts of the spectrum, both of which are indicator of plant photosynthetic activity. Apart from finding the best pre-processing technique, there are new possibilities to fuse the information from multiple spectral normalisation techniques using incremental learning approaches such as sequential pre-processing through orthogonalization (SPORT) (Mishra et al., 2020a, 2020b). In the SPORT, a sequence of partial least-squares models are developed utilising sequential orthogonalization step to learn any new information that is enhance by different spectral normalisation techniques. The SNV, VSN and SPORT can be implemented using the freely available multi-block data analysis toolbox called MBA-GUI (Mishra et al., 2020c).

Cluster centroid shows that the clustering on raw reflectance spectra (Fig. 5A) just captured the differences in global intensities of the pixels. Such differences are the result of illumination differences and do not carry physicochemical information. The clustering on the SNV pre-processed spectra was able to avoid the illumination effects and so

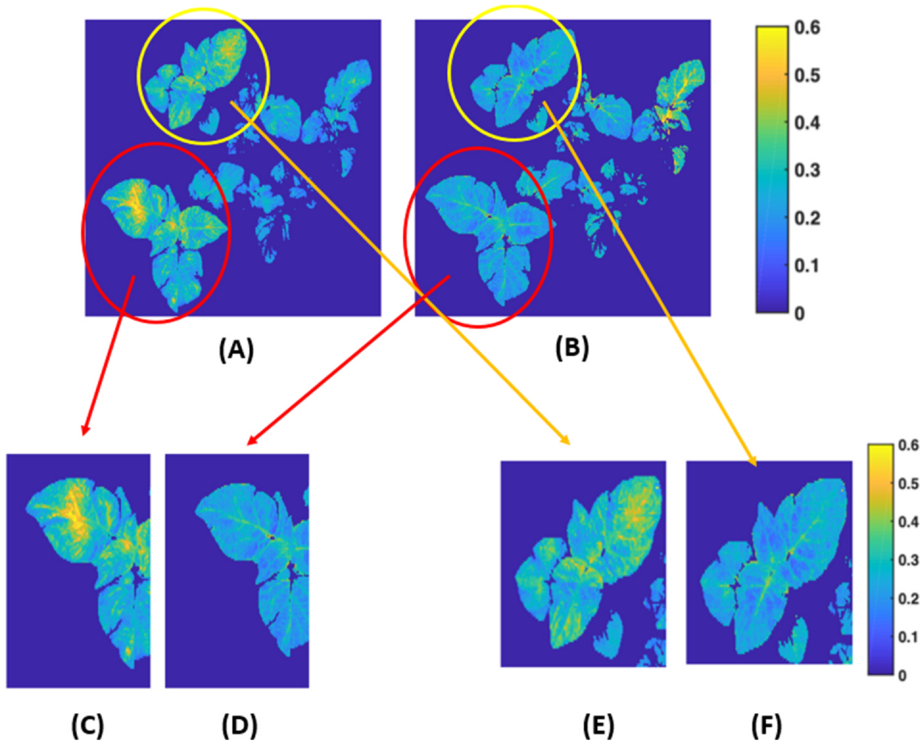

Fig 4. (Reproduced with permission from (Mishra et al., 2020a)) Spectral image corresponding to ~700 nm. (A). Before any correction, and (B). after VSN correction. The illumination effects has been eliminated, zoomed plants (C). plant 1 before correction, (D). plant 1 after correction, (E). Plant 2 before correction, and (F). plant 2 after correction.

captured physicochemical information. However, the information was mainly limited to the color differences. Capturing of the color differences by the cluster centroids of SNV shows that the pre-processed data still has its main variability in the color part of the spectrum, which is masking the chemical differences between the leaf blade and the vein. In the case of the VSN pre-processed data, the clustered centroids have their main differences in the chlorophyll and the red edge parts of the spectrum, both of which are indicator of plant photosynthetic activity. Differences highlighted by VSN in this region can be understood as differences in the photosynthetic activity of the leaf blade and vein which are at the origin of the separate clusters and the segmentation in the cluster maps.

Variations in illumination can also be dealt with via innovative camera hardware and electronics. An example is the use of polarized HSI which can separate the absorption and scattering components without any prior data processing (Xu et al., 2019a,b). Biochemistry prediction can then be performed with the absorption characteristics and the physical parameters can be predicted with the scattering characteristics. However, polarized HSI is currently limited to the laboratory and there are no commercially available systems. Another interesting hardware innovation is light-field hyperspectral cameras such as the 'Cubert ULTRIS (https://cubert-gmbh.com/)'. Light-field cameras capture stereo and hyperspectral data simultaneously. Further, in post-processing, the data can be combined to generate stereo matched hyperspectral images (Zhu et al., 2019). Stereo matched hyperspectral images will capture the information related to the local inclination of the plant leaves, and therefore, can be used to generate directly the 3D hyperspectral model without needing extra sensors or measurements. However, this technology is relatively new and its use for agricultural applications has not yet been demonstrated. In addition, portable HSI systems are emerging (Behmann et al., 2018) but they are less sensitive and tend to suffer to a greater extent from the effects of variations in illumination.

A proper understanding of light interaction with the complex geometry of plants will assist with the development of new approaches to correct for the effects of variation in illumination. Such understanding

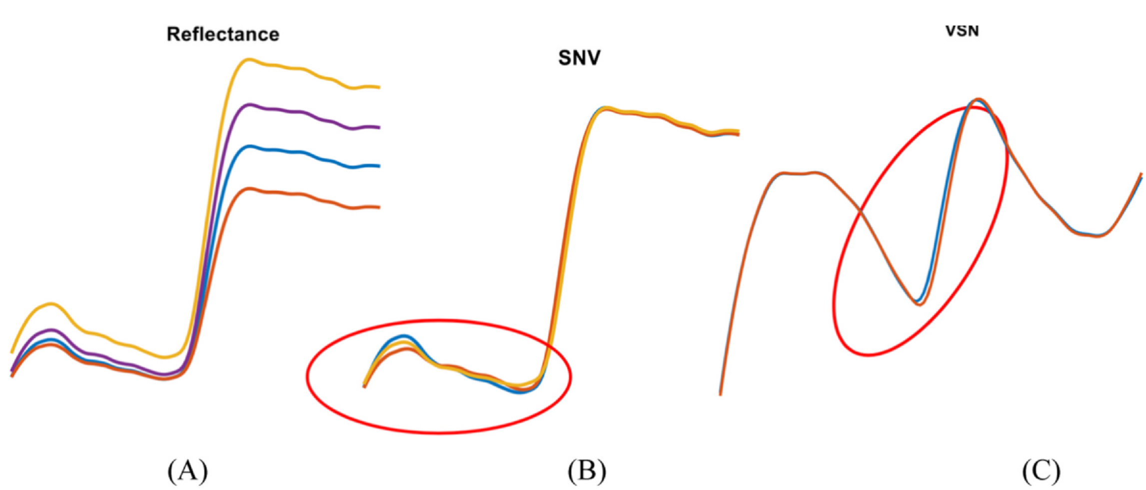

Fig. 5. (Reproduced with permission from (Mishra et al., 2020a)) The spectra clusters for (A). Raw reflectance, (B). SNV pre-processed, and (C). VSN pre-processed.

can be developed with simulation studies. In a work related to wheat canopy simulation using light propagation modelling in the open source platform Open-Alea, Adel-Wheat and a 3D dynamic model of the aerial growth, it was found that the multiple scattering effects present in the imaging scene can lead to underestimation of the property of interest (Al Makdessi et al., 2017). In later work by the same authors, the oblique projection approach was developed which simulates the illumination effects and then removes such effects via spectral projections (Al Makdessi et al., 2019). The results were promising but practical applications are still lacking. Due to recent advances in deep learning and the emergence of concepts like generative adversarial networks (GAN) (Yinka-Banjo and Ugot, 2020), advanced simulations to aid the understanding of the interaction of light with complex plant geometry can also be explored. However, currently there are no applications of GAN in the domain of plant simulation and modelling. Use of GAN to simulate the 3D white reference based on the 3D shape of the plants, which can be used to perform the radiometric calibration in place of the flat white reference, can be foreseen.

6. Conclusions

This work reviewed the recent application of close-range HSI of the whole plant and the approaches to correct the illumination effects. Some of the major conclusions from this review are:

1. Recent applications of HSI of complete plants are mainly for four purposes: monitoring and detection of biotic and abiotic stress; prediction of biochemical parameters; testing of the potency of crop protection compounds; and detection of disease in plants.
2. There is likely no way to (mechanically) avoid the illumination effects in HSI of plants as the light interaction with the complex geometry of plants always lead to phenomenon such as light scattering, shadowing, multiple reflections and a complex mixture of all these effects.
3. Proper illumination effects correction is not only the requirement but is the necessity to get conclusive outcomes from HSI of plants.
4. Currently, six main approaches to illumination correction are available i.e. spectral averaging, spectral normalisation, radiative transfer modelling, oblique projections, the fusion of 3D with hyperspectral data and deep learning.
5. In the perspective of the high-throughput digital phenotyping, spectra normalisation approaches, such as SNV and VSN, are the fastest, easy to implement and model-free approaches.
6. Spectral averaging is the least effective approach as it removes the rich spatial information from HSI data.

In summary, close-range HSI of plants is becoming a key tool for digital phenotyping and is getting widely adopted within high-throughput plant phenotyping setups. Several approaches for illumination correction are now available. In next few years, some new approaches either hardware or software-based are expected to be introduced which would ultimately facilitate the effective application of close-range HSI for plant analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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