



Perspectives in High-Throughput Phenotyping of Qualitative Traits at the Whole-Plant Level

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Abstract

Recent advances in technology have enabled the rapid development of high-throughput automated and semi-automated field and laboratory phenotyping platforms worldwide. In this review, we discuss possible ways of matching the qualitative traits of the above-ground parts of crop plants, also defining the target traits and possible approaches that would be useful in automated phenotyping systems. Optical tools based on light reflectance are presented as a high-throughput and low-cost alternative to some destructive analytical methods. Special attention is paid to hyperspectral imaging and its integration in high-throughput phenotyping systems, as well as its special applications for the assessment of specific plant material traits associated with food quality.

Keywords

Phenotyping · Phenomics · Hyperspectral imaging · Qualitative traits

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Abbreviations

DESI-MSI	Desorption electrospray ionization mass spectrometry imaging
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
FAO	Food and Agriculture Organization of the United Nations
HPLC-MS	High-performance liquid chromatography–mass spectrometry
HSI	Hyperspectral imaging
IR light	Infrared light
LEDI	Lettuce decay indices
MALDI	Matrix-assisted laser desorption ionization
MS	Mass spectrometry
MSI	Mass spectrometry imaging
NIR light	Near-infrared light
NMR	Nuclear magnetic resonance
PCA	Principal component analysis
PMT	Photomultiplier tube
QTL	Quantitative trait locus
RGB camera	Red–green–blue camera
SVDD	Support vector data description
SWIR light	Short-wave infrared light
TIR light	Thermal infrared light
TNC	Total nitrogen content
TOF	Time-of-flight (mass spectrometer)

1 Introduction

At present, there are intense discussions regarding the many problematic challenges that agriculture may face in the coming decades. The United Nations Food and Agriculture Organization (FAO) considers that the world's population in 2050 will be 34% greater than it is today. Currently, 49% of the world's population lives in urban areas, while in 2050 this proportion will be closer to 70% (Alexandratos and Bruinsma 2012). During this time period, climate change and the development of biofuel production will present major risks to long-term food security. Population growth, urban civilization, and climate change can stimulate high plant crop concurrence as possible resources of food, fiber, bioenergetic products, and other industrial needs in future (<http://www.fao.org>). Such resource factors will require new approaches to the genetic and agronomic components of crop production systems (Fiorani and Schurr 2013). Continual management of sustainable water and soil resources and more adequate utilization of genetic diversity are basic steps that are necessary to increase productivity (McCouch et al. 2012; Bakker et al. 2012; Shao et al. 2005, 2009).

Currently, society has the technology and resources to provide long-term food security for all, despite some risks and challenges. In the past few years, many

approaches have been discussed and incorporated into the constantly improving new process of highly automated, non-destructive phenotyping of plants. Plant phenotyping is the extensive evaluation of multiple plant features, such as architecture, cultivation, development, resistance, ecophysiology, and harvest, as well as the analysis of particular significant specifications for quantity analysis of specific biological active compounds. Detailed and specific phenotyping strategies are required to research genome-wide associations and permit high-resolution linkage mapping investigations, and also to create models of genomic selection for plant development. To simplify assessments and understanding of the phenotype based on the metabolome, metabolomics experiments are required to answer questions that underlie genomics, transcriptomics, and proteomics. Three types of metabolomic tools are available. First is a direct tool with which it is possible to identify the spectrum of secondary metabolites. Second is a tool that performs epigenetic gene amplification at the level of the whole phenome, enabling the description of the entire genotype by a metabolome marker characteristic. Third is a tool that identifies a marker for the optimal adaptation of a specific genotype to the environment (Khakimov et al. 2013; Sytar et al. 2016).

Improvements in the specificity and throughput of phenotypic assessment at all biological levels—phenomics, metabolomics, and genetics—are the main objectives of modern phenotyping. Because of their automation, improved data integration, remote sensing, and experimental design, phenotyping systems minimize labor and costs (Cobb et al. 2013; Chen et al. 2002). These systems developed for phenotyping are important for representing the full set of genetic factors that play roles in the phenotypic variation of quantitative parameters for cells, tissues, and organs; these plant phenotyping systems also play roles in identifying stages of development, plant species, and environments (Cobb et al. 2013). The newly developed plant phenotyping platforms produce significantly more data than the initial platforms did, and they need special systems for data management, access, and storage. New statistical tools are needed for enhancing the experimental design and for making greater use of ideas to promote the integration of data, as well as for deriving biologically significant signals from experimental and environmental noise (Cobb et al. 2013). The phenomics field faces two major challenges. First is the features analysis of a large quantity of genetic lines, and second is the replication of measurements of dynamic traits (i.e., traits whose phenotype changes during the vegetation period) (Araus and Cairns 2014). To characterize a genotype, it may be more valuable to determine levels of a few key compounds in several organs and at several time-points, rather than obtaining the full metabolic profile for just one sample. For phenotyping projects that engage several hundred samples, it can be useful to use more oriented, less costly methods for all samples with full-scale metabolomics results. Preliminary metabolomics work might indicate which substances are the main contributors to the phenotype of interest and lead to a focused analysis that is more productive and cost effective for phenotyping than previous methods.

The following high-parameterized platforms for phenotyping have been installed in the field or laboratory (Table 1): (semi)-automatic evaluation of morphometric parameters using red-green-blue (RGB) image analysis; chlorophyll and

Table 1 Automated and semi-automated high-throughput plant phenotyping platforms

Location/Producer	Platform	Features	URL
PSI (Drasov, Czech Republic)	PlantScreen™	Conveyor phenotyping system in controlled environmental conditions with analysis of chlorophyll fluorescence, kinetic and thermal imaging, morphometric and RGB analysis, and hyperspectral and NIR imaging; uses an automated weighing and watering system.	http://www.psi.cz
LemnaTec (Aachen, Germany)	Scanalyzer ^{3D}	Comprehensive non-destructive 2D-3D assessment of plant physiological traits in controlled environmental conditions.	http://www.lemnatec.com
INRA (Montpellier, France)	Phenopsis	Specific platform for phenotyping <i>Arabidopsis</i> plant growth under controlled environmental conditions.	http://bioweb.supagro.inra.fr/phenopsis
INRA (Versailles, France)	Phenoscope	Automated phenotyping device to handle and monitor hundreds of individual pots.	https://phenoscope.versailles.inra.fr
INRA (Montpellier, France)	Phenodyn	Temporal analyses of growth rate and transpiration of hundreds of monocot crop species.	https://www.phenome-fppn.fr/phenome_eng/Facilities/Montpellier-Controlled/PHENODYN
INRA (Montpellier, France)	Phenoarch	Automated platform based on a LemnaTec system to analyze the genetic determinants of plant responses to environmental conditions.	https://www.phenome-fppn.fr/phenome_eng/Facilities/Montpellier-Controlled/PHENOARCH
PhenospeX (Heerlen, Netherlands)	FieldScan	Phenotyping under field- or semi-field conditions that is designed to screen large populations.	http://phenospex.com
WPS (De Lier, Netherlands)	WPS	Fully automated digital phenotyping system using high-throughput RGB sensors.	http://www.wps.eu/en/plant-phenotyping
Keygene (Wageningen, Netherlands)	PhenoFab ^R	Greenhouse service operation that combines phenotyping technology with trait interpretation to exploit phenotypic variation.	http://www.keygene.com/products-tech/digital-phenotyping/
Jülich Plant Phenotyping Centre (Jülich, Germany)	Growscreen	Non-invasive methods designed to quantify shoot morphometric and functional parameters and root architecture.	http://www.fz-juelich.de/ibg/ibg-2/EN/methods_jppc/GROWSCREEN/_node.html

(continued)

Table 1 (continued)

Location/Producer	Platform	Features	URL
Wageningen UR (Wageningen, Netherlands)	PhenoBot	Autonomous mobile robot with camera promises to output direct registered depth and color image for morphometric analysis.	http://www.wageningenur.nl
Wiwam (Gent, Belgium)	Wiwam Conveyor	Integrated robotic system for phenotyping of larger plants with automated irrigation and measurement of a variety of plant growth parameters at regular time intervals.	http://wiwam.be
Australian Plant Phenomics Facility (Adelaide, Canberra, Australia)	PlantScan	Provides non-destructive analyses of plant morphology, structure, and function by using high-resolution cameras with cutting-edge information technology.	http://www.plantphenomics.org.au/services/plantscan/

2D-3D Two-dimensional-three dimensional, *NIR* near infrared, *RGB* red-green-blue

fluorescence kinetic imaging; and hyperspectral, thermal, and near-infrared light (NIR) imagings. These systems are composed of controlled watering and nutrient regimes regulated by automatic weight systems and environmental controls in the imaging cabinet (in laboratory platforms). The control and programming of platform systems, as well as data analysis, is performed with sophisticated and user-friendly software packages. Such newly developed phenotyping systems have tools for assessing many photosynthetic parameters; RGB systems can assess plant morphology, IR-thermal cameras can evaluate stomatal conductance, and hyperspectral imaging (HSI) systems can evaluate the metabolomics of experimental plants at different stages of growth.

Currently, as part of the phenotyping system, HSI is a promising novel technology that requires the development of unique methodologies in order to make use of all spectrum data (Kuska et al. 2015). In this review, we will present information about phenotyping research using HSI and other imaging techniques. For assessing qualitative traits using the phenotyping system, we recommend choosing a plant that has a wide range of metabolites and one that is resistant to some stress factors. Among known crop plants that have been proposed as models for phenotyping research, it is important to choose one that has undergone genetic analysis, because genetic variations, particularly those characterized by fast growth, can be useful for model plants. Improving the breeding process of cultivated plants requires a unified mix of classical breeding approaches, together with one or more categories of phenotyping, in order to identify genotypes with the desired features (Tester and Langridge 2010). Only by clearly specifying and differentiating the physiological phenotyping approach at every breeding step will it be possible to effectively address the challenge of integrating phenotyping into a breeding program. Accordingly, the aim of this review is to identify perspectives in the high-throughput phenotyping of qualitative traits at the plant level.

2 Phenotyping of Qualitative Traits

2.1 Definitions of Target Traits for Phenomics, with Emphasis on Qualitative Traits and the Schema of Leaf Traits

A known limitation of the spectroradiometric approaches used for identifying the developmental stages of plants and for identifying differences in plant architecture is related to the following characteristics of the leaf surface: epicuticular waxes, roughness, and trichomes (Fig. 1). Spectroradiometric approaches can negatively influence the measurement of some features (e.g., biochemical parameters) (Cobb et al. 2013). The effect of leaf anisotropy is more visible in phenotyping systems with remote sensing-based approaches that use reflected rather than transmitted radiation. Furthermore, it is useful to use plants at the same developmental stage in order to standardize measurements. Variability in plant architecture should be minimized because the sun's elevation must be taken into account, as many spectroradiometric evaluations in the field use passive sensors (Araus and Cairns 2014).

Phenotyping platforms can be specific to plant parts and their growth periods (Chen et al. 2014). Phenotyping platforms use model-assisted methods (phenomics, metabolomics, proteomics, and genomics) that are specific to the developmental level and architecture of different plants (Fig. 1).

Genetic diversity in some plants has been described with plant morphology methods, randomly amplified polymorphic deoxyribonucleic acid (DNA), chloroplast and DNA sequence analysis, microsatellite markers, seed protein allozymes, and restriction fragment length polymorphisms (Mensack et al. 2010). Currently, accessible bioinformatics approaches and databases have been used in plants for metabolic quantitative trait locus (QTL) and comparative pathway analyses (Nadella et al. 2012). Genetic loci that affect quantitative features such as biomass or metabolite content can be connected to molecular markers (Fumio et al. 2012). This method

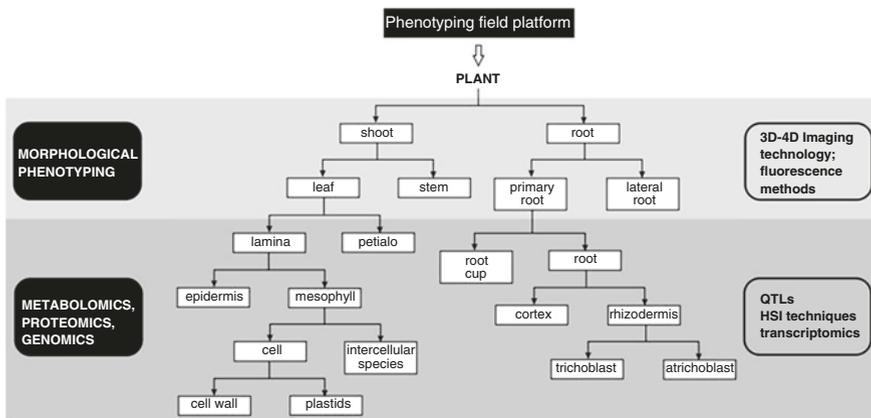


Fig. 1 Definitions of target traits for phenomics, with emphasis on qualitative traits and leaf traits

is called QTL mapping, and it can simplify the identification of new genes in biochemical metabolic pathways. For the QTL mapping of metabolites and for phenotyping experiments, *Arabidopsis thaliana* is a good choice as a model seed plant, because of its highly advanced genetic and molecular characteristics. In mQTL investigations, the total number of genes that have been molecularly estimated remains limited. To improve the network of known metabolic reactions, multi-parallel analytic approaches for the assessment of gene expression, rapid gene identification, and determination of metabolite and protein content would be useful (Brotman et al. 2011).

For example, an analysis of the complete metabolite and phenotype profile that was undertaken with the aim of improving the quality of tomatoes found that nearly 50% of the metabolic loci were combined with QTLs that were associated with whole-plant-yield peculiarities. Correlation analysis established a cartographic network that showed associations with the phenotype of the whole plant and independent relationships with metabolites. This cartographic network also contained information about connections with important organoleptic and nutritional metabolites (Schauer and Fernie 2006). The utility of combining agronomic and metabolomic features of wheat plants was evaluated by a QTL study that aimed to determine potential targets for the improvement of breeding selection. The usefulness of including plant phenotyping in the assessment of the practical features of the wheat mapping population has been confirmed (Hill et al. 2015). In this case, the genetic-metabolic synergism and its connection to crop phenotypes can be used as a target with which to improve breeding selection strategies and efficiency.

2.2 Possible Approaches to Matching Qualitative Traits

Three types of parameters can be evaluated in the phenotype. These are threshold, quantitative, and qualitative parameters. A qualitative parameter can be expressed qualitatively, which means that the phenotype varies by categories that do not need to have an exact form. The pattern is typically influenced by a single gene (monogenic). Plant metabolomic methods have facilitated large-scale analyses that have determined quantitative and qualitative variations of metabolic content in different types of plants (Tang et al. 2014). The genetic factors identified through genetic mapping approaches have been connected to plant variation through the linkage of genetic architecture to the plant metabolome (Brestic et al. 2015). However, in an experiment with rice grains, the content of many metabolites was found to be highly sensitive to environmental factors, but only weakly sensitive to genetic background (Matsuda et al. 2012). A one-line analysis of physiological performance and morphological phenotype features obtained further results that increased our understanding of the complex molecular mechanisms that regulate quantitative parameters (Carreno-Quintero et al. 2013). To improve such investigations, methods allowing the non-destructive, fast, and simultaneous identification of primary and secondary metabolites have been developed with phenotyping systems using HSI.

It is known that many experiments are managed using plants grown under more-or-less controlled environmental conditions (Chen et al. 2013; Yan et al. 2011, 2012, 2013). The goal of many such studies is to make phenotypes of various plant species comparable, in order to study plant changes under a range of suboptimal conditions, or to study genotypes in a specific environment (Poorter et al. 2012.). New approaches are emerging that focus on the traits and phenotyping needed. In novel plant phenotyping, the aim of selecting optimal features rather than directly studying the crop plant yield is related to precise phenotyping in the field or in controlled conditions, or in networks and models of field experiments (Tardieu and Tuberosa 2010). The discovery of complete genomes and the rapid improvement of tools with which to characterize gene expression and proteins complements the already developed objective methods for primary and secondary metabolite detection (Roessner et al. 2002).

It is important to remember that plant metabolism during growth is highly dependent on light parameters and the time of day. Diurnal fluctuation occurs when the levels of some metabolites differ during the day and night (Urbanczyk-Wochniak et al. 2005). Carbon availability can affect the number of metabolic pathways and photosynthesis and respiration rates, which change with light availability. Therefore, special treatment must be used when harvesting samples of plant tissue. In ideal conditions, all sampling would be performed at the same time of day or within a very small time window in order to minimize biological variation. When samples are collected for a metabolomic experiment, it is necessary to quickly suppress metabolism during the extraction procedures and biochemical analysis.

It is necessary to examine the cleanliness of samples harvested for analyses of metabolites (Verhoeven et al. 2006). Contamination of plant tissue with pests (e.g., aphids), bacteria, or fungi can be prevented with the preliminary data analysis during plant growth, especially if the samples are collected from plants under natural growth conditions and not under controlled laboratory conditions. The application of chemicals to control pests such as sciarid flies and aphids in laboratory conditions should also be considered when analyzing metabolites obtained from plants that have received chemical treatment.

In a field experiment performed by Ossipov et al. (2008), frozen samples of leaves were placed directly in liquid nitrogen for transport to the laboratory. Although metabolite analysis can be performed on site, the cost can be problematic. For example, concentrations of carotenoids, anthocyanins, and chlorophylls can be estimated on site from cut leaf discs by spectrophotometry, via basic solvent extraction (Sims and Gamon 2002). Bruneti et al. (2013) provided detailed descriptions of sampling and processing assays and emphasized the importance of *in situ* techniques in field sites. However, in laboratory-based settings, such techniques are more applicable for secondary metabolite analysis.

Many plant metabolomics techniques have used whole plants, organs such as leaves or roots, and tissue parts, which are homogenized until the samples can be extracted. The methodology for tissue analysis should be decided at the start of the experiment, as the methodology can affect the analysis protocol. For example, typically an amount of 10–100 mg of fresh tissue is acceptable for most analytical methods, including gas chromatography, mass spectrometry (MS), and nuclear magnetic

resonance. The heterogeneity of cell types in plant organs is represented by more than 40 different cell types. Normally, any metabolomic analysis of a whole organ shows more than one metabolite and reflects the metabolome of a particular cell type.

Current improvements in plant metabolomics techniques focus on using either laser micro-dissection technology or *in situ* MS. Laser capture micro-dissection technology is used to investigate specific kinds of cells, which are analyzed by common metabolomics approaches. For metabolite imaging, *in situ* MS can be used for a particular tissue. The most recently developed approaches include mounting tissue sections on a matrix-assisted laser desorption ionization (MALDI) plate and exposing them to laser pulses. Molecules are vaporized by the laser and ionized by the matrix prior to being transferred into the mass spectrometer for analysis. The MS analysis characterizes the molecules present in the area that is subjected to laser pulses, which is approximately 50 μm in size. Specific software then allows the deconvolution of individual mass spectra across the tissue and the generation of tissue images (Roessner and Bacic 2009).

2.3 Optical Tools Based on Light Reflectance as a High-Throughput and Low-Cost Alternative to Destructive Analytical Methods

Optical detection approaches adapted to microfluidic biosensors, based on methods such as chemiluminescence, fluorescence, and surface plasmon resonance are being developed (Pires et al. 2014). For actual-time scanning, small-size detection tools have been developed for point-of-use in certain samples. The size of these devices can be reduced using microfluidic chip-based technologies, which combine a few laboratory targets on a separate chip. This methodology addresses the distribution of small volumes of fluids in microchannels (Pires et al. 2014).

Schwarz and Hauser (2001) have used optical and electrochemical constructions for investigating high-sensitivity microfluidic analytical systems. These detailed constructions were later described by Mogensen et al. (2004). Further, for many common biochemical analyses, there have been innovations in micro- and nanomechanical sensors (Waggoner and Craighead 2007).

Optical methods have been used to estimate the sensitivity of the refractive index, light intensity variations, and interference patterns (Fiorani and Schurr 2013). Positive features of optical methods include real-time detection (in the range of hundredths of seconds), a low quantity of required sample, and the ubiquity of these methods in the laboratory. However, limitations include the complexity of the set-up of the optical tools and the expense of conventional optical instrumentation.

Chemiluminescence uses sensor technologies such as microplate readers, photomultiplier tubes (PMTs), charge-coupled device (CCD) cameras, inorganic photodiodes or photoconductors, and organic photodiodes. Fluorescence uses the same CCD cameras, organic photodiodes, and inorganic photodiodes, as well as complementary metal-oxide semiconductor image sensors. Absorbance is measured using a visual sensor, a CMOS image sensor, and a CCD camera. The surface plasmon resonance

sensor opto-microfluidic detection method mostly uses an infrared camera and a CCD camera (Pires et al. 2014).

All conventional optical detection methods have been used in microfluidic biosensors (Jiseok et al. 2013). As long as spectrophotometers, microscopes, lasers, PMTs, and CCDs are precisely linked to Lens Opacity Classification System, such systems are hard to convert to portable detection devices, which are low in price. Rather, with the goal of decreasing the cost of diagnostic platforms, the waveguides and photodiodes used in optoelectronic technology have been profitably joined to microfluidic systems (Baker 2008; Chen et al. 2014). Ultra-sensitive methods for the detection of bio-analytes at nano- and microscales are being developed, and integrated optical microfluidic platforms can be used with these methods (Fahlgren et al. 2015).

Another optical tool is Raman imaging, which has been used to develop an understanding of various real-time pretreatment strategies, while providing integral chemical information (Lupoi et al. 2015). Raman spectroscopy is a spectroscopic technique with vibrational features that are measured as diffused photons, which are produced during the interaction between matter and light. During the development of this methodology, C. V. Raman used sunlight as the light source. Modern Raman spectroscopy techniques use lasers in visible (VIS), ultraviolet (UV), or near-infrared (NIR) spectra. A significant parameter of the Raman phenomenon compared with IR spectroscopy is that molecular compounds are advanced to virtual vibrational, short-lived levels. A molecule is recognized as “active” for Raman spectroscopy when the interchange of light with the molecule creates changes in the polarizability of the electron. The archetypal Raman spectrum would be expected to be C–C, C=C, C–H, C–O, H–C–C, C–O–H, H–C–H, and other vibrational modes (Larsen and Barsberg 2011; Agarwal and Atalla 2010). The choice of the excitement wavelength for obtaining high-quality Raman spectra is difficult, as the Raman intensity is precisely equivalent to the fourth power of the incident frequency.

In recent years, different approaches have been developed to test the difficulty of obtaining Raman spectra for molecules with highly fluorescent capacities, such as lignin. The 1064-nm neodymium-doped yttrium orthovanadate (Nd: YVO₄) or neodymium-doped yttrium aluminum garnet (Nd: Y₃Al₅O₁₂) lasers, which are sources of NIR technologies, can be used in some improved Raman spectra methodologies (Meyer et al. 2011; Lupoi and Smith 2012). Phenolic components define lignin solubilization and reactivity (Zakis 1994). The use of alkaline media results in vibrational lignin modes in pulps, with a concomitant shift expressed in the ionization of the corresponding phenolic lignin model compounds (Warsta et al. 2012). In wood pulp analysis, a low noticeable shift developed because of the lower content of functional phenolic compounds in the pulps compared with that in the model compounds. It was found that the shift appeared to be due to ionization of the phenolic group. Improved band intensities also appeared after the pH increased. However, no shift was detected during the measurement of 3,4-dimethoxytoluene of non-phenolic background.

Mass spectrometry imaging (MSI) underwent an important trial in the mapping of targeted compound distributions in plant tissue (Todd et al. 2001). Recently MALDI-MSI has been used in the direct profiling and imaging of biomolecules in

thin tissue sections (Jackson et al. 2005). Currently, MSI applications can be used to estimate and characterize the proteomes expressed by tissues and organs (Chaurand et al. 2002). The preparation of samples for metabolite analysis by MALDI-MSI is more complicated than that required for proteins or peptides, because metabolites in the sample can be diffuse, can easily circulate via metabolic changes, or can be eliminated during sample preparation (Lee et al. 2012). Organic solvents can be used to help in the estimation of lipids. This has specific implications, as organic solvents are often employed for localizing proteins and peptides in the preparation of biological samples. It is generally recommended to avoid the washing or rinsing steps during sample preparation in order to avoid removing small metabolic compounds in the sample. After harvesting, to avoid metabolite degradation, plant tissue samples should be freeze-dried or rapidly dried in a vacuum desiccator on the imaging plate. For plant IMS applications, it is recommended to use fresh tissue because the effects of histological tissue fixation are not well studied.

Another promising application of high-throughput screening is the droplet-based microfluidic system (Lim et al. 2013). The fluorescence intensity measurements are non-destructive, with photomultipliers or photodiode signal detection, and images are created by high-speed or standard cameras (Millera et al. 2012). Any type of droplet-based microfluidics which uses simple alignment procedures can be combined with microoptics. This combination, by focusing light with a single element, results in high sensitivity and resolution. Higher throughput is attained by lenses combined in an array. This combination can be used for cells and fluorescent particle analysis too, as it may support the creation of high-quality fluorescence signals and a significant reduction of analytical costs.

Despite the development of modern high-throughput methods and sample preparation, the analyses remain relatively costly and laborious. However, non-invasive fluorescence-based methods, which are typically used in plant phenomics and may serve as early steps in metabolomic screening targeted at nutritionally and pharmaceutically important phenolic compounds, are now part of modern research activities. Sytar et al. (2015) presented the results of *in situ* measurements from many plant species, and they indicated high interspecific variability, which seems to be promising for further studies.

In general, the term 'metabolomics' covers two basic approaches. First, the non-targeted approach, which aims to determine as many compounds in the sample as possible. This approach may lead to discoveries of new active molecules, but it is slower and more expensive than the second approach. The second, targeted, approach aims at research for practical applications, such as food or pharmaceutical needs, targeting a single, or relatively narrow, well-defined group of compounds (e.g., amino acids, phenolics) (Verpoorte et al. 2005). Depending on the target metabolites, several possible non-invasive techniques may be used in the early stages of metabolomics research. These techniques are based on the simultaneous assessment of multispectrally induced chlorophyll fluorescence and HSI findings that can be part of the phenotyping system. These techniques, though not yet widely used, have become more popular in the past decade, owing to the introduction of commercially available devices.

3 Hyperspectral Imaging as a Component of Non-destructive Plant Metabolomics

3.1 Principles of the Hyperspectral Method and Basic Information about the Hyperspectral Method

Hyperspectral imaging has been widely accepted as a non-destructive, rapid, and safe method of qualitative analysis, and the method has been used for evaluation of the qualitative traits of a wide spectrum of food products (Elmasry et al. 2012). Hyperspectral imaging has been specifically improved for remote sensing tools (Goetz et al. 1985). Its capacity to consolidate imaging techniques and spectroscopy is its main benefit. Hyperspectral imaging systems have been adapted to make direct synchronous measurements of different components and to identify the spatial distribution of measured components in tested products or plant tissues (Gamalero et al. 2004). The characteristics of molecular structure are related to the absorption, reflection, and transmission of electromagnetic radiation under certain wavelengths (Sun 2010). It is known that UV, VIS, NIR, mid-IR, and far-IR lights are electromagnetic waves. Each region of the electromagnetic waves is linked to a precise type of transition on atomic or molecular levels corresponding to various degrees of repetitiveness. Plant tissues, like any other biological material, may be bound together by various molecular forces and connections. The bonds C–H and O–H are mostly present in water, carbohydrates, and fats. The bonds N–H and C–H are typical for derivatives of petroleum products and organic compounds. Electromagnetic waves are transmitted through a plant sample when it is exposed to light. The energy of the incident electromagnetic wave in the plant samples is modified by the extension and rotational fluctuation of some bonds, such as N–H, O–H, and C–H. Therefore, for the investigation of some parameters and the precise identification of plant examples, transformations in levels of molecular energy can be determined by spectroscopy.

At the macro level, electromagnetic waves are seen as light. Electromagnetic wave transitions are exposed as light reflection or scattering, and also as light transmission. The emission and absorption strength and wavelengths depend on the chemical and physical parameters of the plant material, because the consumed light partly diffuses into the sample of plant tissue. The obtained incident light is transformed to a spectrum and to images converted by hyperspectrometers. Images received from the hyperspectral camera can characterize the plant compounds and the physical characteristics of the plant samples. However, for such characterizations, HSI technology, which produces large datasets, requires improvements in appropriate image processing and multivariate data analysis tools.

3.2 Target Bands and Indices Based on HSI

The efficiency of agriculture crop classification obtained by using hyperspectral narrowband data is substantially higher than that of broadband data analysis (Thenkabail et al. 2004a, b; Schull et al. 2011). In experimental research with

different plants, the optimal hyperspectral narrow bands were identified, based on a comprehensive analysis of the literature that combined classifications of redundant bands (Table 2).

Table 2 Recommended optimal hyperspectral narrow bands for different plants

Waveband interval	Waveband center (nm)	Importance in vegetation and agricultural cropland studies	References
<i>Blue bands</i>			
	466	Chlorophylls: Chlorophyll <i>a</i> and <i>b</i> in vegetation	[1]
<i>Green bands</i>			
	515	Leaf nitrogen	[3]
	510/530	Anthocyanin	[3]
	520/442	β -Carotene	[4]
	550	Chlorophyll and biomass: Total chlorophyll, chlorophyll/carotenoid ratio, vegetation nutritional and fertility level	[4], [5]
	550	Reflectance in the green region of the spectrum proved to be sensitive to anthocyanin content	[6]
	584–609	Determination of total flavonoid content of leaf samples in two colors (green-yellowish and yellow)	[7]
	662–688	Determination of total flavonoid content of leaf samples in two colors (green-yellowish and yellow)	[7]
<i>Red bands</i>			
	675	Chlorophyll absorption maxima: Greatest crop-soil contrast is around this band for most crops in most growing conditions. Strong correlation with chlorophyll <i>a</i> and <i>b</i>	[2]
	682	Biophysical quantities and yield and chlorophyll absorption: Leaf area index, wet and dry biomass, plant height, crop type, crop discrimination. Strong correlation with chlorophyll <i>a</i> and <i>b</i>	[1], [2]
<i>Red-edge bands</i>			
	700, 720	Stress and chlorophyll: Nitrogen stress, crop stress, crop growth stage studies	[1], [8],[9]
	759	Nitrogen	[10]
	778/658	Phytochrome P660	[3]; [11]
<i>Near-infrared (NIR) bands</i>			
	845	Biophysical quantities and yield: Leaf area index, wet and dry biomass, plant height, grain yield, crop type and crop discrimination. Often used in combination with an index from 682 nm	[8]
	845/730	Phytochrome P730	[3]
<i>Far NIR (FNIR) bands</i>			
	1028/2101	Lignin	[3]
	1100	Biophysics quantities: Sensitive to biomass and leaf area index. The point of the most rapid rise in spectra with unit change in wavelength in FNIR bands	[12]

(continued)

Table 2 (continued)

Waveband interval	Waveband center (nm)	Importance in vegetation and agricultural cropland studies	References
	1215	Moisture and biomass: The point of the most rapid fall in spectra with unit change in wavelength in FNIR bands	[13]
<i>Short-wave infrared (SWIR) bands</i>			
	1316	Nitrogen: Leaf nitrogen content of crops	[14]
	1731/1691	Nitrogen	[3]
	1725	Lignin, biomass, starch, moisture	[8], [13]
	1954	Crude fat	[10]
	2211/2400	Cellulose	[3]
	2359	Cellulose, protein, nitrogen: Sensitive to crop stress, lignin, and starch	[1], [9]
	2370	Crude fiber	[10]

References: [1] Thenkabail et al. (2004a); [2] Thenkabail et al. (2004b); [3] Almeida and De Souza Filho (2004); [4] Chan and Paelinckx (2008); [5] Yang et al. (2010); [6] Merzlyak et al. (2003); [7] Shi et al. (2012); [8] Le Maire et al. (2008); [9] Penuelas et al. (1995); [10] Yi et al. (2008); [11]; [12] Abdel-Rahman et al. (2010); [13] Yao et al. (2010a, b)

Spectral data can present much information about the plant parameters. Usually, the dataset is very large and needs to be analyzed by appropriate multivariate and machine-learning methods. Such methods can aid in the extraction of useful information from spectral data and can also avoid multicollinearity (Witten and Frank 2005). For example, both a stay-green mutation and the effect of megadose methylprednisolone treatment on the compositional consequences of *Lolium temulentum* senescence have been studied. The investigated spectral parameters of the leaf tissue were estimated non-destructively and interpreted by various methods. Principal component analysis (PCA) was used to assess differences in leaf reflectance spectra between genotypes, types of treatment, and time points to demonstrate the disruption of pigment metabolism during senescence caused by the stay-green mutation. The stay-green mutation is caused by the inhibition of protein synthesis and by the combination of types of treatment and time points (Gay et al. 2008).

3.3 Integration of HSI in High-Throughput Phenotyping Systems

With increasing options for image capture and open-source analysis tools, the field of high-throughput plant phenomics is poised to enter a phase of fast development. High-throughput image-based phenotyping is described as a technology that can create hundreds of images of plants per day (Messina et al. 2011). Such volumes allow the analysis of mutant populations, the detection of QTLs, and the discovery of genes by the investigation of various environmental factors (Noah et al. 2015).

The phenotyping system PlantScreen™ has components that can capture broad spectral information. A type of imaging methodology in such phenotyping platforms is used for data collection in quantitative studies. This methodology is a complex feature related to growth parameters, increased yield, and adaptation to abiotic or biotic stresses. The imaging techniques include VIS imaging, imaging spectroscopy, fluorescence and thermal infrared (TIR) imagings, plus tomographic (positron emission tomography [PET], magnetic resonance tomography [MRT] and computerized tomography [CT]) and three-dimensional (3D) imaging (Li et al. 2014). Many image-based phenotyping approaches have originated from the use of non-imaging sensors that have been applied in the field, such as thermography point sensors. Other methods have been introduced from the field of remote sensing, such as the satellite-based calculation of spectral indices (Walter et al. 2015).

Greenhouse-based and growth-chamber phenotyping platforms have the advantage of increased experimental cycling and environmental control, but they are often defined by pot growth and the spectrum of environmental conditions that can be measured. Controlled environmental conditions are also well suited for the investigation of root phenotypes (Bucksch et al. 2014; Moore et al. 2013; Topp et al. 2013). In addition to the development of design strategies that support experimentation, camera technologies are needed to obtain important data related to metabolomics and plant physiology.

The target of plant imaging and analysis is to measure the physiological and qualitative development, growth, and other phenotypic properties of plants through automated processes. Hyperspectral imaging camera technology has become relatively inexpensive and universal, and it stands out among a recent surge of high-throughput phenotyping systems that utilize plant imaging to describe data. Standard consumer cameras use silicon-based sensors that are responsive to light wavelengths in the 400- to 1000-nm range. However, color cameras can be used in the 400- to 700-nm range, which is visible to humans with the inclusion of an IR-blocking filter (VIS camera). VIS camera sensor arrays have three color sensors (red, blue, and green) that are used to assess the true color of each pixel. VIS cameras are used to measure the morphological, geometric, and color properties of plants in the phenotyping system (Tessmer et al. 2013; Yang et al. 2014; Neilson et al. 2015). Infrared (IR) cameras detect NIR light and are used for night imaging (Matos et al. 2014). NIR cameras detect NIR and short-wave infrared (SWIR) light in a region that is useful for detecting leaf water content (Seelig et al. 2008). Thermal infrared (TIR) cameras detect long-wave infrared light, which is emitted by leaves at a temperature-dependent intensity (Sirault et al. 2009). Hyperspectral cameras that detect hundreds of spectral bands with nm-level resolution between 350 and 2500 nm are currently being developed to detect plant stress (Mahlein et al. 2013). Additionally, specialized imaging systems can measure chlorophyll fluorescence after excitation (Chen et al. 2014).

For phenotyping platforms, VIS cameras and additional camera systems can accompany color imaging. A disadvantage of VIS and NIR cameras is that the detection sensors are sensitive to relatively broad ranges of the electromagnetic

spectrum, and specific wavelength information is lost in the output image. In contrast, state-of-the-art hyperspectral cameras can measure hundreds of spectral bands between 350 and 2500 nm at nm-level resolution for each image pixel (Mahlein et al. 2012a, b). Hyperspectral imaging is an encouraging approach for the discovery of both abiotic (Römer et al. 2012) and biotic stresses (Mahlein et al. 2012a, b). The images produced by various high-throughput phenotyping platforms and camera types can present challenges for common image processing and trait extraction.

3.4 Recent Advances in the Use of HSI at the Plant Level

Hyperspectral and fluorescent imaging may provide a means to directly and non-invasively detect and quantify secondary metabolites such as flavonoids and terpenoids (Table 3). In 2015, Deepak et al. compared different imaging approaches and conventional chemical analysis by high performance liquid chromatography (HPLC)-MS for the detection of secondary compounds of birch leaf. Clonally regenerated silver birch from three natural Finnish populations (latitudes 60°, 62°, and 66°) and four genotypes from each population growing in the Botanic Garden, Joensuu, Finland (62°35' N, 29°46' E) were used as the plant material. Chemical analysis by HPLC-MS revealed genetic differences within populations from different provenances based on the secondary chemistry. Visible, SWIR, and mid-infrared imagings were used to study spectral reflectance differences in leaf material from individual trees. Fluorescence approaches and UV reflectance imaging at the macroscopic scale were used to localize within-leaf spatial differences for UV-absorbing and fluorescent flavonoids (Deepak et al. 2015).

The following crop plants have been used for phenotyping projects related to breeding strategy and the influence of stress factors (especially drought): rice (Fischer et al. 2003), wheat (Monneveux et al. 2012; Rajendran et al. 2009), barley (Hartmann et al. 2011), maize (Dignat et al. 2013; Winterhalter et al. 2011), sorghum (Schaffert et al. 2011), and pearl millet (Vadez et al. 2012). Some are minor crops that are considered to be high in value but are not widely grown in some countries. It should be noted that a minor use in one country may be regarded as a major use in another country. Thus, many leguminous plants, such as buckwheat, can be minor crops and also *Pseudocereals*. Among leguminous plants, common beans (Beebe et al. 2013), chickpeas (Upadhyaya et al. 2012), cowpeas (Hall 2012), and groundnuts (Sheshshayee et al. 2011) have been used for phenotyping. Clonal plants known for phenotyping include cassava (Okogbenin et al. 2013), sweet potatoes (Vilaró 2011), and bananas (Ravi et al. 2013).

Arabidopsis plants are used as model plants for phenotyping because they have known genotype characteristics and can be easily manipulated with known gene parameters. Additionally, it is easy to identify connections between phenotyping technology, genetic background, and plant metabolomics. The Plant Image Analysis phenotyping system (<http://www.plant-image-analysis.org/>) is a convenient database of commercial and open-source image analysis tools (Lobet et al. 2013). The

Table 3 Application of hyperspectral imaging (HSI) for different plant parts and needs

Plant species	Part of plant	Use of his	References
Birch (<i>Betula</i> spp.)	Leaves	Detect and quantify secondary metabolites such as flavonoids	[1], [2]
Portobello mushroom (<i>Agaricus bisporus</i>)	Whole plant	Food quality evaluation	[3], [4]
Wheat (<i>Triticum</i> spp.)	Kernels	Food quality evaluation	[5]
<i>Arabidopsis thaliana</i>	Whole plant	Detection of ultraviolet (UV)-absorbing secondary metabolites	[6]
Hypericum (<i>Hypericum</i> spp.)	Whole plant	Detection of UV-absorbing secondary metabolites	[6]
Barley (<i>Hordeum vulgare</i> L.)	Leaves	Determining nitrogen levels in plants	[7]
Pepper (<i>Capsicum</i> spp.)	Leaves, whole plant	Determining nitrogen levels in plants	[8]
Barley (<i>Hordeum vulgare</i> L.)	Grains	Food quality evaluation	[9]
Cotton (<i>Gossypium hirsutum</i> L.)	Leaves	Determining nitrogen levels in plants	[10]
Cotton (<i>Gossypium hirsutum</i> L.)	Leaves	Determining chlorophyll concentrations	[10]
Eucalyptus (<i>Eucalyptus</i> spp.)	Canopy	Determining chlorophyll concentrations	[11]
Soybean (<i>Glycine max.</i> L. Merr.)	Canopy	Determining chlorophyll concentrations	[12]
Apple (<i>Malus domestica</i>)	Fruits	Defect detection	[13]
Apple (<i>Malus domestica</i>)	Fruits	Detection of fecal contamination	[14]
Lettuce (<i>Lactuca sativa</i>)	Leaves	Defect detection	[15]
Citrus (<i>Citrus</i> spp.)	Fruits	Detection of fungal infections	[16]
Sugar beet (<i>Beta vulgaris</i> L.)	Leaves	Detection of fungal infections	[17], [18], [19]
Maize (<i>Zea mays</i> L.)	Kernels	Detection of <i>Fusarium</i> damage	[20], [21]
Wheat (<i>Triticum</i> spp.)	Kernels	Detection of <i>Fusarium</i> damage	[22], [23]
Maize (<i>Zea mays</i> L.)	Kernels	Determination of mycotoxin contamination	[24], [25]

[1] Deepak et al. (2015); [2] Matros and Mock (2013); [3] Gaston et al. (2010); [4] Taghizadeh et al. (2011); [5] Jaya et al. (2010); [6] Hölscher et al. (2009); [7] Pytela et al. (2015); [8] Yu et al. (2014); [9] Arngren et al. (2011); [10] Zhao et al. (2005); [11] Coops et al. (2003); [12] Ferri et al. (2004); [13] Baranowski et al. (2012); [14] Yang et al. (2011); [15] Simko et al. (2015); [16] Lorente et al. (2013); [17] Mahlein et al. (2010); [18] Mahlein et al. (2012a, b); [19] Hillnhütter et al. (2011); [20] Williams et al. (2010); [21] Williams et al. (2012); [22] Shahin and Symons (2011); [23] Bauriegel et al. (2014); [24] Firrao et al. (2010); [25] Yao et al. (2010a, b)

majority of open-source tools found in the Plant Image Analysis database (Lobet et al. 2013) focus on the phenotyping of plants with rosette architecture (*Arabidopsis thaliana*) and specific plant organs such as excised leaves, although some experiments were conducted with HSI analysis (Table 3).

Spectral reflectance is also known as a fast method for determining nitrogen levels in plants. The general principle of spectral analysis involves reflectance values measured at different wavelengths. Nitrogen content is predicted from the linear dependence of reflectance and reference values of leaf nitrogen content. Reflectance spectra were determined with the PolyPen RP400 (Photon Systems, Drasov, Czech republic) on the second and third youngest leaves of barley after 10 days of nitrogen treatment, as an average value for each nitrogen nutrition group. An average reflectance spectrum in the interval of 500–650 nm was negatively linked to the dose of nitrogen added to the medium. A similar degree of correlation was found when reflectance spectra were related to the weight of shoot biomass or the nitrogen content of the leaves. The reflectance parameter green normalized difference vegetation index, determined from the second leaves of individual plants, was dependent on the nitrogen content in the respective plants, with linear regression showing $r^2 = 0.612$ (Pytela et al. 2015).

Mass spectrometry-based imaging techniques can also be used as part of HSI to provide knowledge of proteins and other metabolites in plant tissues. Graphs of different types of compounds can be made from primary and secondary metabolism findings. Differential dissemination diagrams have been created for some compounds, particularly lipids, sugars, and amino acids, and for polyphenols, anthocyanins, glucosinolates, and alkaloids as secondary metabolites from different plant varieties (Matros and Mock 2013). Matrix-free laser desorption/ionization-MSI was used at the single-cell level to analyze the allocation of secondary metabolites with UV-absorbing capacities in *Hypericum* species (phloroglucinols and naphthodianthrones) and in *Arabidopsis thaliana* (kaempferol derivatives) (Hölscher et al. 2009). The preparation of samples and choosing of matrix substances and function is important for imaging analysis. The parameters are critical for obtaining images of sufficient quality using MALDI-MSI; also, desorption electrospray ionization (DESI)-MSI, combined with the above-mentioned imaging advances, provides strategies for integral applications. Molecular ions caused by MALDI time-of-flight (TOF), ESI-TOF mass spectrometry, and objective metabolite analysis were used to detect fructans, hexoses, and sucrose accumulation during the development of barley grain (Peukert et al. 2014).

The early detection of decay, and its gradual development, have been assessed in lettuce plants using chlorophyll fluorescence and hyperspectral imaging techniques. Two lettuce decay indices (LEDI) for the detection of leaf tissue decay have been proposed. The background of one of the indices (LEDI₄) is based on three identified hyperspectral imaging wavelengths. Chlorophyll fluorescence imaging was used for establishing the other index (LEDI_{CF}). Both indexes, LEDI₄ and LEDI_{CF}, when analyzed on yellow, light green, dark green, green, and red leaves, demonstrated near 97% validity in classifying tissues as being withered or fresh (Simko et al. 2015).

The typical green leaf reflectance spectrum is in the range of the VIS (400- to 700 nm) and NIR (700- to 1000 nm) wavebands for hyperspectral analytical approaches. Few classes of spectral variables have been found to be valuable for describing the way in which reflectance spectra shift owing to changes in pigment concentrations. However, the concentrations of pigments and spectral variables have shown empirical relationships. An alternative technique for HSI analysis

aimed at estimating the quantity of pigments has been established in canopy radiative transfer models and in various model versions of the physical leaf (Blackburn 2007). The high absorption spectral ranges have a high sensitivity to low concentrations of pigments, whereas reflectance can be more sensitive to high concentrations (Yamada and Fujimura 1991).

The presence of chlorophyll is the major feature that encompasses the relation between anthocyanin content and reflectance (Sytar et al. 2017a). Two narrow wavebands with reflectance ratios from the VIS, red edge, and NIR regions were selected for the assessment of chlorophyll in leaves (Zhao et al. 2005) and also at the canopy range (Coops et al. 2003; Ferri et al. 2004). The establishment of universal chlorophyll indices in plant reflectance data is necessary to cover more sources of spectral variability by using several narrow wavebands (Thenkabail et al. 2000). The calibration of reflectance data in a more detailed predictive model for a specific plant type is possible using just two wavebands. To resolve the problem of chlorophyll reflectance and anthocyanin identification, an index that uses the green narrow band (550 nm) and the reflectance of the early red edge band (700 nm) has been suggested (Gitelson et al. 2001).

Hyperspectral imaging has shown high effectiveness for assessing the quality of fruit and vegetables and their safety regarding surface defects, contamination, starch index, bruises, sugar content, freeze damage, firmness, and bitter pit (Nicolai et al. 2007; Lorente et al. 2012). Defect detection with HSI analysis is based on identifying the spectral trait wavelengths for the defect and then using these spectral parameters to create discrimination models. Thermal imaging (3500–5000 nm) was integrated with HSI (400–2500 nm) to detect bruises created 1 h before the assessment of bruising (Baranowski et al. 2012). Hyperspectral imaging has been adopted for the automatic detection of the early stages of fungal infections in post-harvest citrus fruit. To choose the optimal parameters for the determination of decay in citrus fruit generated by *Penicillium digitatum* and *Penicillium italicum* fungi, a receiver operating characteristic curve has been proposed that considers 74 parameters, covering 57 purely spectral variables and 17 spectral indexes (Lorente et al. 2013).

The detection of fecal contamination of vegetables and fruits is necessary because feces can contain different pathogens. An HSI system developed in 2011 by Yang et al. (2011) used a camera with electron multiplying CCD technology, which is able to achieve high-resolution images at high speed under low illumination. Fluorescence methods that could identify almost 99% of the fecal spots were used at wavebands of 680, 684, 720, and 780 nm (Yang et al. 2012).

To detect insect damage, classification models have been established using various methods of analysis—quadratic discriminant, linear discriminant, a support vector data description (SVDD) classifier for stepwise partial least squares (PLS) discriminant analysis, and a distribution-based Bayesian discriminant model. HIS has revealed better detection capacity for fruit quality and safety analysis compared with the standard investigation (Wu and Sun 2013).

Determination of moisture content and surface color is also needed for the assessment of fruit and vegetable quality. Current color assessments have been regularly achieved by the collection of images from a hyperspectral camera in

reflectance mode in the spectrum area between 400 and 1000 nm, which also covers the visible spectrum (Taghizadeh et al. 2011). Color was predicted in two ways; the first method used the calculation of tristimulus values from the hyperspectral images, and the second used a multivariate PLS regression algorithm (Taghizadeh et al. 2011). The capacity of HSI to determine color was similar to that of a colorimeter (Ariana and Lu 2010), and HIS seemed to obtain more information about the surface than that obtained from RGB camera images (Taghizadeh et al. 2011).

Since 1998 many HIS studies of grain analysis have been conducted, consisting of the identification of sound or stained grains, analysis of grain color, determination of grain kernel vitreousness, and wheat class bias (Jayas et al. 2010).

HSI has been used to classify crop seeds, including maize, barley, rice, groats, oats, soybeans, and wheat seed. An NIR spectrum range (900–1700 nm) HSI system was used for mathematical modeling to analyze early pregerminated barley in order to separate low- and high-quality barley kernels (Arrgren et al. 2011). The model used a direct classification structure established on the basis of traits of insensitivity to kernel orientation.

Toxicogenic fungi in seeds are toxic for both animals and humans. HSI systems in the VIS and short-wave NIR (400–1000 nm) zones or long-wave NIR zones (960–1662 nm and 1000–2498 nm) have been used for the indirect detection of *Fusarium* damage in maize (Williams et al. 2010) and wheat (Shahin and Symons 2011), as well as for the investigation of fungal expansion (Williams et al. 2012). The symptoms of *Fusarium* disease are easily identified by the analysis of hyperspectral images. Therefore, this methodology can help to map the presence and assess the level of *Fusarium* infection. From this point of view, independent field harvests can be considered. *Fusarium* detection in wheat is established by HSI and chlorophyll fluorescence methods, according to the requirements, parameters, and limitations of the specifications of these methods. The adjustment of spectral changes caused by fungal infection is detected by HSI. The low physiological activity of tissues which occurs under the influence of *Fusarium* is the basis for confirmatory factor analyses (Bauriegel and Herppich 2014).

Measurement of the quantitative parameters of mycotoxin infection is a specific topic in the qualitative analysis of seed and grain production. Of note, HSI has been used to detect fumonisin contamination produced by *Fusarium* spp. in milled maize (Firrao et al. 2010). HIS has also been used to determine the concentration of aflatoxin in corn kernels after their inoculation with *Aspergillus flavus* spores (Yao et al. 2010a, b). Multiple linear regression was adopted to calculate correlations ($r^2 = 0.44$ for fumonisin analysis and $r^2 = 0.72$ for aflatoxin analysis).

In 2010, Gaston et al. used HSI to predict polyphenol oxidase activity in mushrooms; PCA was used for data analysis. The results led to the probability of establishing a sensor that could rapidly detect mushrooms that have a high possibility of developing enzymatic browning. Certainly, the discussed research covers the utility of HIS in quality management and food industry safety (Gaston et al. 2010).

Based on the full spectra of HSI and the important wavelengths when using the Dumas combustion method, quantitative relationships between spectral data and the total nitrogen content (TNC) in plant organs (root, stem, and leaf), and also in whole

plants, were separately determined with the help of PLS regression. The PLS regression model, constructed using the relevant wavebands for the estimation of TNC in whole plants, showed a promising correlation coefficient for prediction and the root mean square error for prognosis. Each TNC pixel within the region of interest of the pepper plant sample was evaluated to make a structural distribution map of the TNC. These accomplishments indicate that HSI is encouraging and presents a powerful potential tool with which to estimate the structural distribution of nitrogen content in plants (Yu et al. 2014).

HSI at the leaf level has proven to be specifically relevant for the estimation and quantification of fungal invasion. Each fungal infection affects sugar beet tissue in a specific form, and the resulting spectral reflectance shows fungal infection-specific spectral markers. Mahlein et al. (2010) and Hillnhütter et al. (2011) have characterized similar effects for foliar and soil-borne infections of sugar beet, using non-imaging hyperspectrometry.

HSI has high potential as a non-destructive diagnostic tool for disease detection. In 2012, Mahlein et al. investigated spectral reflectance and the peculiarities of leaves diseased with *Cercospora* at different stages of development. Light microscopy was used to characterize changes in the host tissues, at the morphological level, caused by colonization with the pathogen. An HSI line scan spectrometer (ImSpector V10E, Specim Spectral Imaging Ltd., Oulu, Finland), which has a spectral resolution of 2.8 nm from 400 to 1000 nm, was used under controlled conditions for the extended monitoring of infection syndromes at the time of the pathogenetic process. Pixel-wise mapping in the VIS and NIR areas for spectral reflectance allowed the detailed characterization and detection of disease at the leaf tissue level. The leaf architecture was related to the leaf patterns of spectral reflectance. The separation and identification of symptoms in regions showing all stages of ontogenesis, from youth to maturity, can assist in mapping classification. Better knowledge of leaf reflectance changes induced by plant diseases was achieved via pure spectral markers pixel-wise extraction using HSI. The process of pixel-wise extraction noticeably raises the specificity and sensitivity of hyperspectrometry in the immediate sensing of plant infection and developing plant disease symptoms (Mahlein et al. 2012a, b).

3.5 Future Perspectives of HSI at the Plant Level

Quantitative studies of plant systems and functions support the better adaptation of plants to conditions of low-input agriculture and resource-limited qualitative breeding processes (Fabio and Ulrich 2013). Hyperspectral imaging is a component of multidisciplinary research on plant phenotyping, and it focuses on features that can help in genotype selection with expanded efficiency. Hyperspectral imaging technology is evolving rapidly; however, parallel achievements in large-scale phenotyping applications, detailed coverage of minimum points of information regarding protocols for different experiments, and integration with modeling and data management schemes are required.

Hyperspectral imaging was basically advanced for remote sensing and is currently available as an efficient analytical approach for the non-destructive investigation of plants and food. Methods of using HSI techniques, with different sensors for hyperspectral reflectance imaging and fluorescence imaging, and hyperspectral transmission imaging techniques for plant process monitoring are being developed (Gowen et al. 2007). Currently, these HSI techniques are mostly used for food quality control and less for studying plant metabolomics and phenomics.

The HSI system is a valuable piece of emerging technology for the evaluation of food quality, and it can also be used for some commercial needs. It is foreseen that future methodological and technical improvements in HSI systems that suit the pharmaceutical industry will lead to the development of low-cost systems that are also suitable for food industry utilization. Comparatively lengthy times are needed for hypercube image addition, processing, and classification, as well as for other defining factors (Chen et al. 2002). The acquisition time can range from 2 to 4 min, built upon on the resolution of image and target size, but processing and classification times are closely related to computer hardware and software capacities. However, further improvements are needed in phenotyping system constituents, such as faster hardware in cameras and more detailed and efficient algorithms, which can shorten processing time and enable real-time HSI quality monitoring. It is important to develop HSI methodology for the theoretical needs of plant physiology, with the main goal being the creation of a standard spectrum database for the identification of as many biochemical compounds as possible. Using an HSI system as part of the phenotyping system will help to support the complex analysis of plant reactions to different stressors, which could be useful for building promising models of plant behavior under changing environmental conditions (Sytar et al. 2017b). Thus, it is time to develop HSI techniques and methods for the food and pharmaceutical industries, while at the same time building background HSI methodology for plant sustainability and biodiversity as the main goal in plant-level phenotyping.

4 Conclusions

In this review, we have presented information regarding the use of HSI in plant phenotyping. Hyperspectral imaging techniques have been used in experiments on food quality, evaluations of fruit defects, and the detection of fungal infections, as well as the identification of fecal contamination and *Fusarium* damage. The determination of chlorophyll concentrations, mycotoxin contamination, plant nitrogen levels, and UV-absorbing secondary metabolites is part of the metabolic analysis that is performed with non-destructive HSI methods. Hyperspectral imaging cameras can be used to assess qualitative traits in the phenotyping system. The methodology for analyzing biochemical compounds, other than those mentioned here, with HSI cameras requires further development.

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