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REVIEW

Digital image-based plant phenotyping: a review

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Abstract

With the current rapid growth and increase in the world's population, the demand for nutritious food and fibers and fuel will increase. Therefore, there is a serious need for the use of breeding programs with the full potential to produce high-yielding crops. However, existing breeding techniques are unable to meet the demand criteria even though genotyping techniques have significantly progressed with the discovery of molecular markers and next-generation sequencing tools, and conventional phenotyping techniques lag behind. Well-organized high-throughput plant phenotyping platforms have been established recently and developed in different parts of the world to address this problem. These platforms use several imaging techniques and technologies to acquire data for quantitative studies related to plant growth, yield, and adaptation to various types of abiotic or biotic stresses (drought, nutrient, disease, salinity, etc.). Phenotyping has become an impediment in genomics studies of plant breeding. In recent years, phenomics, an emerging domain that entails characterizing the full set of phenotypes in a given species, has appeared as a novel approach to enhance genomics data in breeding programs. Imaging techniques are of substantial importance in phenomics. In this study, the importance of current imaging technologies and their applications in plant phenotyping are reviewed, and their advantages and limitations in phenomics are highlighted.

Keywords: image analysis, phenotyping, phenomics, plant breeding

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Introduction

The global demand for nutritious food and crops is growing rapidly. In the coming decades, it will be a major challenge for farmers and breeders to meet the food demands of an increasing world population, which is likely to reach 9 billion by 2050 (Araus and Cairns, 2014). Satisfying the needs of the world food population will require acceleration in plant breeding programs to produce increased potential crop yields (Baker, 2008). Therefore, agricultural researchers and plant breeders should focus on the application of genomics and novel technological innovations to enhance existing plant breeding programs to develop plants with higher biomass, greater nutrition, and higher resistance to biotic and abiotic stresses. Remarkable advancements in genetic tools and techniques have resulted in the development of high-yielding, stress-tolerant plants via genetic selection (Badr et al., 2002). However, the lack of accessibility to phenotypic information hinders advancement in this field. Farmers and plant breeders have been making selections based on phenotypes before the discovery of DNA and molecular markers (Araus and Cairns, 2014). They have learned and observed that superior genetic changes may only be identified via a series of crosses among breeds and cultivars under different environmental conditions. Recent advances in high-throughput phenotyping have provided more rapid and cheap genomic information and have led to the development of large mapping populations and diverse panels of thousands of recombinant inbred lines. However, inexpensive genotyping has limited phenotyping in gene discovery and the development of molecular markers.

Currently used phenotyping tools and techniques cannot offer adequately rapid and precise measurement of traits of a large number of lines; therefore, they have become an impediment to crop breeding and development. Existing phenotyping technologies can be considered sluggish, costly, labor-intensive, and mostly destructive (Furbank and Tester, 2011). Plant function and performance are directly affected by the nature of the genome and the characteristics of the surrounding environment. Growth, development quality, and productivity are all functions of light, temperature, climate events, soil characteristics, plant diseases, insects, weeds, and other factors. Constraints can be efficiently monitored by employing different imaging tools and techniques (Sankaran et al., 2015).

Imaging techniques include thermal infrared imaging for the detection of water stress, chlorophyll fluorescence imaging for quantitative analysis of photosynthesis, near-infrared spectroscopy for identifying physiological changes in plant organs caused by nutrient deficiencies, visible–near-infrared hyperspectral imaging for shoot biomass estimation, and shortwave infrared hyperspectral imaging for water absorbance. The images captured by the sensors are analyzed with advanced image processing software tools to acquire pixelwise phenotype data of various phenotypic traits of plants (Munns et al., 2010). The objective of the current review is to explore the importance of imaging techniques and recent developments in plant phenotyping under controlled conditions and to discuss the potential application of imaging techniques in plant phenomics while highlighting their advantages and limitations.

Importance of modern plant phenotyping

Plant phenotyping is the comprehensive assessment of complex plant traits such as growth, development, tolerance, resistance, architecture, physiology, ecology, yield, and the basic measurement of individual quantitative parameters that form the basis for more complex traits (IPPN, 2020). With the rapid advancement of sequencing tools and techniques, the whole genome of various plant types is now accessible in databases online worldwide. Arabidopsis genome sequencing is a breakthrough in plant genomics (Weigel and Mott, 2009). Currently, many essential crop varieties have been sequenced and interpreted (Heffner et al., 2009). However, the full interpretation of genetic data for genomic analysis still requires significant effort.

Traditional phenotyping tools are time-consuming, costly, and destructive in certain phenological stages. The purpose of existing phenotyping is to increase the accuracy, precision, and interpretation of phenotypes at whole levels of living organism structures while decreasing the expense and labor cost via automation, remote-sensing, enhancing data integration, and experimental design. The 'omics' approach includes genomics, proteomics, transcriptomics, and metabolomics (Chen et al., 2014). 'Omics' combined approaches have further potential in assisting crop breeding programs, resulting in a new approach (phenomics) in which high-throughput analysis of various features of an organism is addressed.

The phenomics notion has changed all practices in crop research and development and is described as the characterization of the full set of phenotypes in a given species. In genomics, a sequenced genome is completely characterized, whereas, in phenomics, we cannot characterize the whole phenome because of its highly dynamic nature and multidimensional properties. However, it is possible to perform high-throughput phenotyping on a set of certain traits. Throughput in plant phenotyping refers to the number of individual units at certain structural levels in plants. Dimensionality addresses the various phenotypic characteristics of different spatial and temporal systems, for instance, the composition of plants, physiology, and performance. Similarly, dimensionality covers the number of genotypes, and the diversity of environmental circumstances and treatments are considered at the time of phenotyping (Dhondt et al., 2013).

Genotype-phenotype mapping together with the important speed of trait discovery greatly enhances phenotypic assessment and prediction (Topp et al., 2013). Quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS) are helpful means for genetic analysis, providing vital data about genomes in different plant studies. They are widely responsible for gene mapping (Badr et al., 2002; Yin et al., 2004). The availability of widespread phenome data allows plant relationships to be explored throughout the entire population. As a result, phenomics research to a greater extent entirely characterizes potential phenotypes, creating the organizational biological and performance-linked attributes under various environmental circumstances in a given genotype.

Frequently used imaging tools and techniques in phenomics

Plant phenomics can be employed in crops under controlled conditions in greenhouses and glasshouses as well as under field conditions. The environmentally-controlled greenhouses and glass-houses are equipped with various automatic control procedures to monitor and record ambient factors (temperature, relative humidity, light, and water content) in plants which are ideal for plant phenotyping (Basak et al., 2019). Also, there are some essential tools and techniques involved in plant phenomics. Imaging and image processing methods with illumination sources from the visible to near-infrared range offer nondestructive plant phenotype image datasets. These methods have enhanced the accuracy, increased the throughput, and yielded high-dimensional phenotype information for modeling and prediction of plant growth and development (Tardieu and Tuberosa, 2010; Golzarian et al., 2011).

The use of integrated image-based new tools and techniques in phenomics and environmentally controlled facilities and platforms have resulted in better performance and offer a novel perspective for enhancing plant phenotypes (Granier and Denis, 2014). Plant phenotyping based on imaging techniques is ideal for integrating various phenotypic protocols; they allow researchers to create possible genetic characteristics and understand the multiple monitoring networks of primary adaptive phenotypic differences on a group of plants with high-throughput genome sequenced quantitative research (Furbank and Tester, 2011). Plant phenotyping platforms based on imaging and high throughput have resulted in widespread tools for plant biology, supporting the plant phenomics domain (Paproki et al., 2012). Several different imaging technologies (Table 1), such as visible, infrared, fluorescence imaging, and imaging spectroscopy, are currently being exploited to acquire high-throughput, multidimensional phenotypic information from plant organs to a molecular scale in a few minutes (Fiorani and Schurr, 2013; Sozzani et al., 2014). Meanwhile, imaging techniques are the main tools in plant phenomics. The ultimate aim is to measure the quantitative phenotype through interactions between plants and light sources such as reflected, absorbed, and transmitted photons. The imaging techniques mentioned have all been broadly used in the plant sciences domain because of their inexpensive cost and simplicity of operation and maintenance.

Table 1. The application and limitations of imaging techniques for plant phenotyping (Adapted from Fiorani and Schurr, 2013).

Imaging	Growing	Application	Limitations
Visible imaging	Controlled environment	Growth dynamics, shoot biomass, yield traits, panicle traits, root architecture, imbibition and germination rates, leaf morphology, seedling vigor, coleoptile length and biomass at anthesis, seed morphology, root architecture	Only provides plant physiological information no spectral calibration; Only relative measurement; shadows and sunlight can result in under or over exposure and limit automatically processing image
	Field	Imaging canopy cover and canopy color; color information can be used for green indices; the use of 3D stereo reconstruction from multiple cameras or viewpoints allows the estimation of canopy architecture parameters	Difficult to analysis complicated whole-shoot of non-rosette species; Pre-acclimation conditions required
Fluorescence imaging	Controlled environment & Field	Photosynthetic status, indirect measurement of biotic or Abiotic stress	Difficult to measure at the canopy scale, because of the small signal to noise ratio, though laser-induced fluorescence transients can extend the range available, while solar-induced fluorescence can be used remotely
Thermal imaging	Controlled environment	Surface temperature; Stomatal conductance water stress induced by biotic or abiotic factors	Imaging sensor calibration and atmospheric correction are often required; Sound physics-based results interpretation needed
	Field	Stomatal conductance; Water stress induced by biotic or abiotic factors water content composition parameters for	Imaging sensor calibration and atmospheric correction are often required; Changes in ambient conditions lead to changes in canopy temperature making a comparison through time difficult, necessitating the use of reference Difficult to separate soil temperature from plant temperature in sparse canopies, limiting the automation of image processing Sensor calibration required, cost large image data

Infrared thermal imaging

The infrared thermal imaging technique is commonly used to quantify and analyze plant growth and development by screening the internal molecular motions of objects that emit heat radiation (Lee et al., 2019). This imaging technique changes heat radiation from an object with an absolute temperature greater than 0 K into digital image data that can be employed to compute the surface temperature of that object. Furthermore, infrared (IR) thermal imaging has been defined as a suitable technique for monitoring the water status of various crops (Vadivambal and Jayas, 2011; Taghvaein et al., 2012), such as olives (Egea et al., 2017), vines (Garcia-Tejero et al., 2016), almonds (Garcia-Tejero et al., 2012) and citrus (Garcia-Tejero et al., 2011). This technique works according to the leaf energy balance. As soon as water stress circumstance takes place plants act in response with a limited stomatal closure, decreasing the stomatal conductance restraining the transpiration by leaf and helping in reduction of the evaporative chilling procedure, causing in high leaf temperature (Jones, 2004). The benefit of IR imaging technique is that offer spatial resolution and more accurate measurement in varying ambient situations (Li et al., 2014) and the downside of IR imaging in the field is that environmental parameter will effect on spatial and resolution of obtained images.

Visible light imaging

In agricultural research in plant science, visible-light imaging is extensively used because it is the simplest and most inexpensive imaging technique. This imaging technique is typically performed by utilizing traditional color cameras with wavelengths ranging from 400 to 750 nm in the electromagnetic spectrum. Imitating human vision enables two-dimensional (2D) imaging to analyze different phenotyping traits and to monitor and record variations in plant biomass (Tackenberg, 2007). Under controlled conditions, glasshouse, greenhouse, and screen house visible imaging techniques are very useful in assessing leaf biomass, crop traits, panicle traits, inhibition and growth rates, leaf physiology and structure, seedling strength, coleoptile length, and biomass at the anthesis kernel morphology and root structural mechanism (Li et al., 2014).

Fluorescence imaging

Fluorescence imaging techniques are commonly utilized in both experimental laboratories and plant fields. This imaging method provides detailed information regarding the plant metabolism circumstances that could be acquired through artificial excitation of plant photosystems and reveals the related reactions (Li et al., 2014). Fluorescence imaging is based on charge-coupled device (CCD) sensors that are sensitive to fluorescence signals, where the signals are generated by illuminating the samples with ultraviolet light.

Usually, the two types of fluorescence are from the red to the far red region and from the blue to the green region. These are produced by ultraviolet light covering from 340 to 360 nm and are a fundamental principle of multicolor imaging techniques. This method provides concurrent capture of fluorescence radiation and offers a rapid method for investigating photosystem II status *in vivo*. The illumination energy absorbed by green plants faces one of the three following consequences: Carbon assimilation, heat dispersion, or fluorescence (Maxwell and Johnson, 2000). As a general rule, the fluorescence imaging system consists of a light source for similar illumination of the targeted surface, a fluorescence sensor, and a computer to regulate data collection and analysis. Usually, blue light is employed for the excitation of chlorophyll fluorescence.

Monochrome CCD sensors are employed to obtain fluorescence images. Fluorescence imaging techniques are mainly used for the early detection of biotic and abiotic stress responses before a decline in growth and development could be measured (De Smet et al., 2012). Moreover, the fluorescence imaging technique provides a strong diagnostic means to resolve the dissimilarity problem of leaf photosynthetic performance and is used in various areas of plant physiology (Siebeck et al., 1997). The majority of fluorescence imaging uses are constrained to the shoot level or the particular leaf of prototypical crops. However, developing dynamic software and improving common techniques for fluorescence image phenotyping, processing, and data interpretation is needed.

Imaging spectroscopy

The application of imaging spectroscopy for plant phenotyping is very promising. It originates from the study of remote sensing of vegetation and measures the interaction of solar radiation with plants (Kakaly et al., 2009). Spectral measurements of the electromagnetic spectra can be acquired by multispectral or hyperspectral sensors, which have the potential to scan wavelengths of interest at regular intervals (Fiorani and Schurr, 2013). Multispectral and hyperspectral measurements of the absorption waveband in the infrared range are widely used to explain different water statuses that assess the canopy water content (Cabera-Bosquet et al., 2012).

Recently, spectral imaging has gain popularity in agriculture field and food industry, for qualitative and quantitative analysis of agricultural materials and food products (Ning et al., 2018; Joshi et al., 2019), as well as for screening and monitoring the vegetative status of plant crops and pathogen detection nondestructively, since, it provides rapid analysis and concurrent spatial and spectral information for a given sample (Seo et al., 2019). A good example of spectral measurement is the extraction of a number of reflectance vegetation indices from simple differences between two wavelength reflectances to normalize the reflectance values. The acquired reflected spectra have important information regarding plant structure and vigor conditions that can be used to assess growth and development characteristics. Thus the application of this imaging technique has broadened its applicability in outdoor fields (Lee et al., 2018). Apart from visible and infrared imaging techniques, hyperspectral imaging can divide images into wavelengths, thereby offering specific parts of the electromagnetic spectrum of the images (Yang et al., 2013). Integrating imaging spectroscopy with aerial platforms makes it suitable for field phenotyping, but the price of the spectral sensor and related structures are reasonably costly.

Tomography and other imaging techniques

During the past decade, magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography scanning (CT), and optical three-dimensional (3D) tomography and imaging technologies have advanced and been introduced to enhance proper visualization of plants. MRI scanners utilize powerful magnetic fields, electric field gradients, and radio waves to produce images of various organs (Li et al., 2014). PET produces 3D images by identifying a pair of gamma rays released by a positron-emitting tracer introduced into the plant. It is utilized to assess photosynthetic functioning and environmental stress and mainly focuses on physiological changes (Baker, 2008). Tomographic imaging can accurately monitor and screen the physiological performance of plants. Likewise, computing tomography (CT) is another useful imaging technique widely used in the medical field to explore the internal structures of the human body. However, in recent years, application of CT imaging technique demonstrated its applicability in agriculture field for quality control and inspection of numerous agricultural material and food products such as Mango (Barcelon et al., 1999), carrot (Donis-Gonzalez et al., 2016) chestnuts, (Donis-Gonzalez et al., 2012). CT uses a precisely collimated beam of X-rays to scan one specific area of an object at a time with more sensitive sensors and reconstruct the object in the 3D image (Ray et al., 2012). The disadvantage of CT is its high cost and long scanning time.

However, the aforementioned tomographic imaging techniques have low throughput, and their image segmentation and reconstruction needs to be further improved for high-throughput plant phenotyping. Nevertheless, imaging techniques can display signs and symptoms at the early stages for a broader range of stresses, improving effective discrimination between usual stresses by using multiple sensors that monitor various physiological processes; for instance, water stress and nitrogen deficiency can decrease chlorophyll concentration, but water stress usually has a rapid effect on stomatal closure, given that only water stress results in leaf wilting. Water stress will inhibit photosynthesis by the stomatal limitation of CO_2 uptake, which will impact chlorophyll fluorescence emission. The dynamics of chlorophyll fluorescence emission will probably vary between water stress and nitrogen deficiency. Visualizing the dynamics of stomatal variability to interpret the dissimilarity of stomatal and perhaps relevant photosynthetic responses requires multi-sensor phenotyping style platforms (Chaerle et al., 2009).

Image analysis approaches

In an easy and simplified manner, the image analysis methodology process (Fig. 1) in plant phenotyping follows the following steps:

1. Image acquisition is the primary step in image processing and utilizes the bulk of image data in image analysis software.

2. Image preprocessing techniques are the subsequent steps that include the use of filters to reduce noise or increase sharpness.

3. Image segmentation entails dividing the images into objects of interest, and the objects are exempted from the analysis process.

4. Image description involves quantifying object features such as area, height, and width.

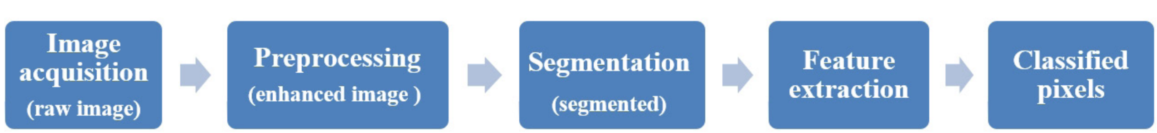

Fig. 1. Flowchart of the image acquisition process.

Advantages of image-based phenotyping

Image-based phenotyping has numerous advantages. These include the following: (a) The methodologies are nondestructive. (b) The same crop plant can be imaged in a sequence throughout its life cycle. (c) A whole plant, plot, or even an entire field can be included in a single image. (d) Quantification of numerous characteristics can be obtained by analyzing a single image. (e) The imaging techniques use near-infrared and infrared spectral data to reveal traits that cannot be assessed by the human eye.

Disadvantages and limitations of image-based phenotyping

The limiting factors for widespread adaptation of image-based phenotyping techniques are the high cost of the equipment (hyperspectral cameras, drones, and controlled environment chambers), the complexity of data analytics software, and the huge amount of data that is generated, which requires superfast computing power with huge storage capabilities.

Plant phenotyping platforms

By integrating the advancement in sensing tools, automation techniques, and aeronautics, computing power has advanced environmentally-controlled-based phenotyping platforms and field-based platforms in recent years. Environmentally controlled phenotyping platforms have been produced or developed in various educational and research institutes in growth chambers and houses. These phenotyping platforms are mainly aimed at research on small plants such as Arabidopsis (De Vylder et al., 2012) and preliminary crop plants. These platforms mainly focus on the deep measurement of plants by integrating robots and image analysis with environmentally controlled structures. However, the application of controlled environments to exemplify environmental conditions has eminent limitations. Small spaces in greenhouses or growth chambers usually do not allow plants to produce flowers and seeds. Consequently, it is impossible to evaluate the impacts of stresses during the reproductive process. Moreover, the amount of soil volume used for plants in a controlled environment is typically less than that of plants in the field, which will affect growth, development irrigation, and nutrient uptake procedures (Akhter et al., 2018). Also, providing responses related to field conditions in a controlled environment is not effective. For example, in greenhouses or growth chambers, light, wind speed, and evaporation amounts are usually less than they are under open-air conditions.

Mostly, breeders and researchers have concentrated on field-level enhancements in yield productivity or abiotic stress resistance or tolerance that supports field-based phenotyping. Field-based phenotyping platforms are known as the sole means for delivering the necessary throughput in terms of the number of plants or populations and a precise explanation of characteristic expression in the real world (White et al., 2012).

Field-based platforms consist of ground-based and aerial-based methods. Ground-based phenotyping platforms include modified vehicles and remote sensing sensors, which have great potential because it allows wider-area and real-time data acquirement for sensing plant circumstances and has some useful instruments such as remote sensing tools, global positioning system (GPS), and geographic information system (GIS) for exploring spatial changeability (Kang et al., 2018), they are known as ‘phenotowers.’ Recently, various types of phenotowers have been developed; for instance, a unique triticale has been developed that can carry eight sets of sensors, two 3D flight cameras. an RGB camera, three laser distance sensors, a spectral imaging camera, and two light curtain imaging structures. It can collect data on the height of plants, fresh weight density, amount of moisture content, growth stage, and tiller density nitrogen content of all plots, having the ability to screen almost 250 plots per hour (Busemeyer et al., 2013).

Aerial-based phenotyping platforms are frequently used as a good alternative to overcome the difficulties of ground-based phenotyping platforms. Aerial-based phenotyping platforms provide rapid quantification of many plants and plots within a matter of seconds. The primary aerial-based phenotyping platforms utilize small airplanes to safely obtain the low speeds needed for high-resolution images at low altitudes. The existing generation of aerial-based phenotyping platforms varies greatly in terms of payload, price, maintenance, and control cost.

Unmanned aerial platforms are alternative blimps that can regularly carry up to 2 kg payloads and have two sensors fixed for simultaneous imaging. Unmanned aerial platforms have greater flight control and autonomy and are becoming increasingly inexpensive. They can be maneuvered into a suitable position and can acquire high-resolution images depending on the sensor. The independence and area noticeable by unmanned aerial platforms are greater, and most unmanned aerial platforms carry an RGB camera and a thermal sensor.

Multipurpose platforms that collect a huge quantity of image data require high-speed computers and a huge amount of data storage for plant phenotyping. Analyzing and managing these data pose another informatics challenge, particularly when projects become large in scale, and so many researchers are engaged. Because a single image has the potential to produce several phenotypic descriptions, these parameters add more to the complexity of subsequent data analysis (Billiau et al., 2012). A research laboratory data management system can handle data storage retrieval and accessibility, ensuring rapid data access to all phenomics researchers (Pieruschka and Poorter, 2012).

Conclusion

In this review, we evaluated various imaging techniques for plant phenotyping. Structural features, deep knowledge dynamic software, and image analysis channels are all very important for image sensors that are engaged in assisting in the measurement of phenotypic data. Visible imaging for the assessment of plant shoot biomass and growth characteristics in two dimensions has been utilized for crops during breeding. Fluorescence imaging was applied to detect foliar infections and thermal imaging was employed for water detection status in plants. Imaging spectroscopy requires standardized methodologies for obtaining the spectral attributes to decrease the amount of raw data in plant phenotyping. Data collection using MRI and PET, the two common phenotyping techniques, is laborious, and more development in software tools is needed to acquire a desirable outcome. There is a great difference in the consistency of imaging techniques in environmentally controlled growth chambers and fields. This variation inconsistency should be kept in mind to understand data collection fundamentals and experimental strategy and proper calibration of imaging-based systems and sensors. Furthermore, with the modification of existing imaging tools and techniques and the development of novel approaches, a large amount of fresh information will be available to help speed up phenotyping.

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