MINI REVIEW

Review on plant phenotyping

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To ensure that crop production keeps up with population growth, it is necessary to select crops with high yields and stress tolerance. It is possible to improve agricultural production to meet the needs of a growing population by establishing a link between genotype and phenotype. In order to establish the connection between a gene and a trait, phenotyping is just as crucial as genotyping. Breeding programs have significantly improved plant phenotyping. The phenotyping of plant stress is progressing steadily, and destructive, low-throughput phenotyping techniques and technologies are being replaced by non-intrusive, high-throughput ones. Several high-throughput phenotyping platforms are currently being used to phenotype various traits associated with biotic and abiotic stress in various crops. Ground-based and aerial-based methods make up field-based platforms. Plant phenotyping, on the other hand, is still developing rapidly. We actually need to create greater limit, execute the new advancements flawlessly into the work process of clients in rearing and the scholarly world, foster legitimate access open doors, and lay out information the executives frameworks that permit information trade and data gain across establishments, areas, and tests. As a result, the technologies and significance of phenotyping for crop improvement were the primary focus of this review paper.

Key Words: *Phenotyping; Genotyping; Selection; Trait; Gene*

INTRODUCTION

To ensure that crop production keeps up with population growth, it is necessary to select crops with high yields and stress tolerance. It is possible to improve agricultural production to meet the needs of a growing population by establishing a link between genotype and phenotype. In order to establish the connection between a gene and a trait, phenotyping is just as crucial as genotyping.

A crop phenotype is the observable characteristics that are expressed as a result of a genotype's interaction with the environment. Plant phenotyping is the wide-ranging assessment of complex plant traits like growth, development, tolerance, resistance, architecture, physiology, ecology, yield, and the basic measurement of individual quantitative parameters that form the basis for more complex traits. The plant phenotype includes these complex traits, and examples of their direct measurement parameters are the root morphology, biomass, leaf characteristics, fruit characteristics, yield-related traits, photosynthetic efficiency, and biotic and abiotic stress response [1].

Selection is a crucial step in plant breeding, and phenotypic selection is the foundation of conventional breeding. Breeders can advance their breeding lines in yield trials and make desirable selections for generational segregation thanks to appropriate trait phenotyping. The primary difficulties of field phenotyping in plant rearing remember estimating great many plots for various conditions while considering accessible assets, time expected for estimations, nature of information gathered, and so on collect and examine data. In this regard, the high-throughput phenotyping offers the chance to upgrade determination force, further develop choice precision, and further develop choice emotionally supportive networks. Platforms for high-throughput phenotyping have been used in greenhouses or growth chambers. Several high-throughput phenotyping platforms are currently being used to phenotype various traits associated with biotic and abiotic stress in various crops. To evaluate plant growth and performance, these platforms make use of robotics, precise environmental control, and imaging (hardware and software) technologies. As a result, the technologies and significance of phenotyping for crop improvement were the primary focus of this review paper [2].

LITERATURE REVIEW

Plant phenotyping

To ensure that crop production keeps up with population growth, it is necessary to select crops with high yields and stress tolerance. It is possible to improve agricultural production to meet the needs of a growing population by establishing a link between genotype and phenotype. In order to establish the connection between a gene and a trait, phenotyping is just as crucial as genotyping.

A yield aggregate is the noticeable qualities that are communicated because of a genotype's collaboration with the climate. The broad evaluation of complex plant traits like growth, development, tolerance, resistance, architecture, physiology, ecology, and yield as well as the fundamental measurement of individual quantitative parameters that serve as the foundation for more complex traits is known as plant phenotyping. The plant phenotype includes these complex traits, and examples of their direct measurement parameters are the root morphology, biomass, leaf characteristics, fruit characteristics, yield-related traits, photosynthetic efficiency, and biotic and abiotic stress response [3].

The workflow for phenotyping as (image capture → data storage and curation → trait extraction → machine learning/classification → models/apps for decision support) has to be carefully designed and efficiently executed to minimize resource usage and maximize utility.

Phenotyping in plant selection and crop improvement

Selection is a crucial step in plant breeding, and phenotypic selection is the foundation of conventional breeding. Breeders can advance their breeding lines in yield trials and make desirable selections for generational segregation thanks to appropriate trait phenotyping. Food crops with desirable characteristics were selected by early farmers and used as seed for subsequent generations, accumulating traits over time. Since the fundamental principles of genetics have only recently been established, phenotyping was the only alternative to experience-based selection at the time. The collector's eye, hand, or invasive, *i.e.*, destructive methods, are typically used to measure various plant properties like structure and function [4-6].

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The primary difficulties of field phenotyping in plant rearing remember estimating great many plots for various conditions while considering accessible assets, time expected for estimations, nature of information gathered, and so on collect and examine data. In this regard, the high-throughput phenotyping offers the chance to upgrade determination force, further develop choice precision, and further develop choice emotionally supportive networks. High throughput phenotyping platforms have been deployed in growth chambers or greenhouses. These platforms utilize robotics, precise environmental control and imaging technologies (hardware and software) to evaluate plant growth and performance [7].

The various methods of plant phenotyping generate a lot of data and are difficult to process. While one piece of this challenge is the non-uniform information designs and absence of similar principles across stages, the more basic part is the shortfall of skill in the more organically arranged research bunches deciphering the information. More advanced sensor and camera systems will be paired with complementary measurements (such as destructive analyses), enabling more detailed screenings and more parameters being measured in a higher spatiotemporal resolution, *i.e.,* more images per time and more detailed images.

Phenotyping for different targets

Plant phenotyping has advanced significantly as a result of breeding programmes. The research into plant breeding aims to produce cultivars through breeding that are resistant to pests and diseases, drought-tolerant, and better suited to low-input agriculture and resource-constrained environments. Plant pressure phenotyping is growing consistently, and damaging, low throughput phenotyping methodology and innovations are being supplanted by non-meddlesome, high throughput strategies. Several high-throughput phenotyping platforms are currently used to phenotype various traits associated with biotic and abiotic stress in various crops. Right now, RGB and multispectral sensors are chiefly used to gauge crop level, biomass and other agronomic attributes under typical and stress conditions. Through controlled phenotypic and genotypic studies, scientists are able to identify and predict heritable traits thanks to the phenotypic parameters, which include not only morphological data but also a large number of physiological and biochemical data as well as deeper mechanistic data [8].

Phenotyping for abiotic stresses: Phenotypic, biochemical, and physiological changes are strongly correlated with abiotic stresses like salinity and drought.

Phenotyping for drought stress: Drought is one of the most critical problems in agriculture due to irregular but frequent changes in rainfall accompanied by increasing temperatures. Numerous attempts have been made to create drought resistant cultivars. Due to the lack of an accurate, high-throughput phenotyping method and the fact that traditional crop phenotyping methods are labor-intensive, time-consuming, and subjective, not many drought-tolerant cultivars have been released. It appeared that many high-quality data could now be collected when automatic High-Throughput Phenotyping (HTP) platforms were introduced to phenomics. Plants' Relative Water Content (RWC), root diameter, root depth, and root hair development all contribute to drought tolerance, as do physiological changes (Stomatal closure, chlorophyll deficiency, photosynthetic rate) that significantly reduce pod numbers and yields at reproductive growth stages like flowering. During drought stress, high-throughput phenotyping makes it possible to collect data and track changes over time. There are various kinds of HTP apparatuses to survey the dry season resilience of harvests. Plant growth rates under drought stress can be predicted using RGB cameras, making it possible to examine entire plants or specific parts of them. Color analysis can also confirm drought stress-induced leaf wilting and chlorophyll deficiency. Based on Bai, et al. multi-sensor systems with portable spectrometers and RGB web cameras were used in 2016 to evaluate crop canopy attributes from soybean and wheat field plots [9]. The transformation of plants under drought stress can be confirmed using Near-Infrared Imaging (NIR). The absorption rate is the highest among the various absorption bands in the spectral range of 1400 to 1450 nm and has a strong correlation with plant moisture content. Invisible early signs of dehydration can be detected with hyperspectral imaging. The warm imaging technique can gauge temperature-related highlights, for example, water content, happening rate, and stomatal conductance through model-based assessment.

Phenotyping of salt stress tolerance: Soil salinity is a major stressor affecting agriculture globally. In saline soils, plants accumulate ions in their shoots, affecting plant growth by ionic toxicity, such as reducing the rate of photosynthesis. During the early stages of salt stress, before ions accumulate significantly in the shoot, an osmotic phase of salt tolerance occurs, termed as shoot ion-independent tolerance. During this stage, growth reduction is due to reduced leaf emergence and expansion. Plant salinity tolerance is assessed using the shoot ion-independent tolerance index. Salinity stress impacts many developmental phases and decreases root length, leaf area, biomass, and total chlorophyll content. Various physiological and metabolic processes are also affected by osmotic stress and ionic imbalance caused by impaired ability of Reactive Oxygen Species (ROS) detoxification, reduced stomatal aperture, and differences in antioxidant enzymes. Ion toxicity is activated by a high accumulation of Na^+ and Cl^- ions in plant tissues exposed to high saline conditions and results into severe ionic imbalance. Image analysis using high-throughput screening is helpful for genetic and physiological studies to develop salt-tolerant crop plants. Recently, a hyperspectral imaging technique was used to assess different genotypes of okra (*Abelmoschus esculentus* L.) for selection of salinity tolerant crop [10].

Phenotyping for biotic stresses: Plant biotic stress phenotyping is an important parameter to predict crop losses caused by different biotic stresses. It can be used to identify superior disease and stress tolerant genotypes as well as to evaluate disease management decisions. High throughput phenotyping systems are designed to measure plant health and productivity; they have obvious applications for measuring food damage caused by herbivores. Defoliation by caterpillars, chlorosis and necrosis caused by aphid infestation, and feeding scars caused by thrips have all been quantified digitally using images captured with RGB cameras or flatbed scanners. For disease identification, prior visual assessment often identifies plant disease symptoms (such as lesions, cankers, blight, rot, or wilt.) or these are based on visible signs of pathogen (urediospore of *Puccinia* spp.) mycelium formation. Early detection is a challenge since plant-pathogen interactions initially cannot be recognized by RGB cameras. However, in controlled environments, pre-symptomatic recognition has been done by hyper spectral imaging, e.g. in *Cercospora beticola* infection in sugar beet. Pioneering work was done by Bravo, et al. by mounting a spectrograph (460-900 nm) on spray-boom height to *Puccinia striiformis* (yellow rust) in wheat and showed that four selected wavebands could distinguish disease at a level of 4 to 5% infection with 96% accuracy [11]. Ultimately, the goal should be to be able to predict disease before it is visual to the naked eye.

The effects of biotic stress on plant physiology are generally heterogeneous, both spatially and temporally. Chlorophyll fluorescence imaging is a powerful tool for exploiting photosynthetic activity at the cellular, leaf and whole plant scales, enables the phenotyping of plants [12].

DISCUSSION

Phenotyping technologies and functionality

Combining the development of sensor tools, automation techniques, aeronautics, and 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 primarily aimed at studying small plants such as *Arabidopsis* and preliminary crop plants. These platforms mainly focus on the deep measurement of plants by integrating robots and image analysis with environmentally controlled structures [13].

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 clarification of characteristic expression in the real world.

Ground-based and aerial-based techniques make up field-based platforms. Ground-based phenotyping platforms are made up of modified vehicles and remote sensing sensors. These platforms have a lot of potential because they can get real-time, wide-area data about plant conditions and have useful tools like remote sensing tools, GPS, and GIS for studying spatial changeability, and they are referred to as phenotowers. As of late, various kinds of phenotowers have been created [14].

The utilization of Unmanned Aerial Systems (UAS) has enabled the rapid development of field high-throughput phenotyping for crops. A variety of sensors, including thermal imaging, Light Detection and Ranging (LiDAR), multispectral imaging (several wavebands), hyperspectral imaging (hundreds or even thousands of wavebands), and regular Red, Green, and Blue (RGB) cameras, can be mounted on an Unmanned Aerial Vehicle (UAV) to gather remote sensing data during field-scale trials. Using this method, plant traits, such as yield, biomass, height, and leaf area index, can be non-destructively estimated.

Electromagnetic radiation is considered to carry the detected information. Electromagnetic interaction (absorption, reflection, emission, transmission, and fluorescence) of healthy plant vary from that of stressed plant. Those properties, which cannot be visualized through naked eyes, are detected with the assist of imaging techniques. The key features of different imaging techniques are described below [15].

Visible light imaging: In agricultural research in plant science, visible light imaging is widely used as it is the simplest and cheapest 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. Imitation of human vision allows Two-Dimensional (2D) imaging to analyze different phenotyping traits and to monitor and record variations in plant biomass. Under controlled conditions, glasshouse, greenhouse, and screen house visible imaging techniques are very helpful 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.

Fluorescence imaging: The artificial excitation of the plant photosystems and observation of related responses can be used to gather information about plant metabolism. Fluorescence can be defined as light emitted when radiation of shorter wavelength is absorbed. Fluorescence is a phenomenon, when light is re-emitted by molecules after they have absorbed radiation at the ultraviolet, visible, and near-infrared spectral wavelengths. Irradiation of chloroplasts with actinic or blue light leads to some remission of the light absorbed by the chlorophyll. Because the use of modulated fluorescence requires significant power for rapid illumination, fluorescence imaging is often used in a controlled environment. The proportion of re-remission light compared with the irradiation depends on the plant's ability to metabolize the harvested light. The re-emitted light is the fluorescence, and it is a good indicator of the plant's capacity to assimilate actinic light. Furthermore, combining an actinic light source with brief, saturating blue pulses can be used to measure the plant's efficiency of photo-assimilation, non-photochemical quenching, and other physical plant parameters [16].

Thermal imaging: Thermal imaging is used to measure leaf surface temperature in order to study the water relations, and specifically for stomatal conductance, since the main determinant of leaf temperature is the rate of evaporation or transpiration of leaves. Abiotic or biotic stresses often result in decreased rates of photosynthesis and transpiration and, the remote sensing of the leaf temperature by thermal imaging can be a reliable way to detect changes in the physiological status of plants in response to different biotic and/or abiotic stresses. Infrared radiations are visualized using thermal imaging; indicating an object as fluorescing part, when blue actinic light temperature across the object's surface. The spectral range of thermal imaging cameras is 3-14 μm and the most commonly used wavelengths are 3-5 μm and 7-14. The transmission of infrared radiation in the atmosphere at these two wavelength ranges is close to the maximum. The thermal sensitivity of smaller wavelengths (3-5 μm) is greater than that of higher wavelengths (7-14 μm), because smaller wavelengths have higher energy than longer wavelengths. However, longer wavelengths can be used to target objects at longer distances; wavelengths between 8 and 14 μm will reduce the error rate from the atmospheric absorption of infrared radiations. In a study led by Giuseppe, thermal infrared imaging was used to distinguish 92 different maize genotypes to screen for drought adaptation in maize. There was an average temperature difference of more than 2°C between the different genotypes under water stress [17].

Imaging spectroscopy: The application of imaging spectroscopy to plant phenotyping is very promising. It comes from the study of remote sensing of vegetation and measures the interaction of solar radiation with plants. 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. 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 [18].

For qualitative and quantitative analysis of agricultural materials and food products, spectral imaging has recently gained popularity in the food industry and agriculture as well as to search and monitor the vegetative status of crops and detect pathogens in a non-destructive way, as it provides rapid analysis and concurrent spatial and spectral information for a given sample. The extraction of a number of reflectance vegetation indices from simple differences between two wavelength reflectance to normalize the reflectance values is a good example of spectral measurement. The acquired reflected spectra have significant information about 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 apart from visible and infrared imaging techniques, hyperspectral imaging can divide images into wavelengths, thus offering specific parts of the electromagnetic spectrum of the images. 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 [19].

Tomographic imaging by MRI, PET or CT: In the past decade, Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), Computed Tomography (CT) scanning, and optical Three-Dimensional (3D) tomography and imaging technologies have advanced and been introduced to improve good visualization of plants. MRI scanners use powerful magnetic fields, electric field gradients, and radio waves to generate images of various organs. PET produces 3D images by recognizing a pair of gamma rays released by a positron-emitting tracer introduced into the plant. It is used to assess photosynthetic functioning and environmental stress and mainly focuses on physiological changes. Tomographic imaging can precisely examine and screen the physiological performance of plants. Similarly, Computing Tomography (CT) is another helpful imaging technique extensively used in the medical field to explore the internal structures of the human body. However, in recent years, application of CT imaging technique confirmed its applicability in agriculture field for quality control and inspection of many agricultural material and food products such as mango and carrot. Computing Tomography (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. The drawback of CT is its high cost and long scanning time [20].

CONCLUSION

Plant phenotyping has speedily emerged in the past decades and generated many new opportunities addressing the various demands, in which phenotyping is needed. In any case, the improvement is as yet advancing quickly. We still need to produce more capacity, implement the new technologies seamlessly into the workflow of users in breeding and academia, develop proper access opportunities, and establish data management systems that allow data exchange and information gain across installations, locations, and experiments. This wants to be done in parallel with continued implementation of novel technologies. Plant phenotyping has come a long way, but there are still a lot of challenges and opportunities ahead. Furthermore, to accomplish a true sense of "cost-effective phenotyping", the trade-off between phenotyping technique investment and manpower cost should be noted, which is primarily dependent on the different objectives.

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