High-Throughput Phenotyping: Enabling Precision Breeding for Plant Improvement

Abstract

Plant improvement involves a huge data collection and analysis, which is laborious and time consuming. For the development of genomics data, there are numerous high-throughputs DNA sequencing methods available, but the ability to provide high-quality phenotypic data is far behind. Collecting phenotypic data from large populations is significantly hindered by the use of manual measurements, which are time-consuming, labor-intensive, and incorrect in traditional approaches for plant phenotyping. An automatic system which helps in the accurate and timely measurement of different traits and its analysis is the need of the hour. It is then met by the high throughput phenotyping, which enables the precision breeding for plant improvement. High-throughput phenotyping, on the other hand, provides distinct benefits that allow for quick, non-destructive, and high-throughput detection, thus overcoming the drawbacks of conventional techniques. This helps in connecting phenotype to genotype and in efficient selection of the genotypes. It provides different platforms for data collection, analysis and storage, useful for sustainable research.

Keywords: *Precise Data Collection, Less Laborious, Efficient Selection*

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I. INTRODUCTION

The term “Phenotype” comes from the Greek word, phainein and typos (meaning show and type, respectively), which was characterized by Wilhelm Johannsen in 1911. Phenotype refers not only to the size, shape, colour like observable characteristics but also, the physiological and biochemical behavior of plants. Recording observations in a small population is possible, whereas the precise measurement of data in large number of plants is tedious and time consuming. However, acquisition of large-scale phenotypic data has become one of the major bottlenecks hindering crop breeding. The main aim of plant breeding programmeis to develop the best performing genotypes with high yielding traits which requires large scale screening of different genotypes. Nevertheless, recent technological advances provide us potential solutions to relieve this bottleneck and to explore advanced methods for large-scale phenotyping data acquisition and processing in the coming years. Because of improvements in a variety of technologies, including sensors, information technology (IT), and data extraction, systems integration, and falling costs, it is now possible to regularly and non-destructively evaluate morphology and physiology across entire populations, throughout stages of development (Houle *et al.*, 2010). In thisHigh-throughput phenotyping, which facilitates non- contact and dynamic measurements, has the potential to offer large scale, high-quality trait data. This opened a new subject of discussion called ‘phenomics’, which denotes the use of high throughput technologies including automation and artificial intelligence-based technologies to enhance the data acquisition, storage and easy processes. This chapter explains a brief about phenomics and different high throughput phenotyping tools, its components and their uses in agriculture and crop improvement.

II. PHENOMICS

In world, three main challenges occur in current agriculture. There are climate change, resource depletion, and population growth. To meet the challenges of global food security in the changing climatic scenario, it would be most imperative to enhance crop productivity under resource competence. The plant research community needs accurate phenotyping to help plants to adapt to resource-limiting environments and low-input agricultural systems (Pieruschka and Schurr 2019) and one of the major challenges is large-scale screening of crop performance as a consequence of its genetic makeup.

According to David Houle *et al.*, (2010) phenomics defined as the acquisition of multidimensional phenotypic data in an organism as a whole. The word "phenomics" was coined by Steven A. Garan at a guest lecture he gave at the University of Waterloo in 1996. Phenomics, the study of the phenome, is a rapidly emerging area of science which aims at characterizing phenotypes in a rigorous and formal way and links these traits to the associated genes and gene variants (alleles) (Close *et al.,* 2011). Phenomics technology can be used to study plants from the small scale, i.e., individual cell, leaf, or plant to the large scale, i.e., ecosystem. Phenomics is the science of largescale phenotypic data collection and analysis. The "-omics" -technologies like genomics, transcriptomics, metabolomics, to analyze the plant performance in the field and further link it to the core molecular genetics. The science of phenomics speeds up phenotyping by using automated high-tech sensors, imaging systems, and computing power (Kushwaha *et al.,* 2024). Depending on the trait under observation, phenomics techniques can be used to characterize large number of lines/ individual plants accurately in a fraction of time, it has few advantages over manual phenotyping, viz., reduced time, reduced labor, and cost involved (Furbank, 2009). The high speed of phenomics based plant phenotyping accelerate the process of selecting plant varieties/ germplasms that perform better in the field under drought, salinity, or hightemperaturestress condition or crops with highphotosynthetic efficiency or those which can performbetter under higher levels of atmosphericcarbon dioxide.

2.1 Forward Phenomics

It is phenotyping tools to "sieve" collections of germplasm for visible and valuable traits. It is used to find out the best germplasm suitable for a particular trait. It also speeds up phenotyping of large number of plants (in case of mapping population) or germplasm lines using automated imaging technology which leads to identification of interesting trait/plant suitable to particular situation. Thousands of plants are grown in pots pre-labeled with barcodes and screened for interesting traits by automated imaging system (Furbank and Tester 2011). The selected plants with the target traits can then be grown up to produce seed for further analysis and breeding.

2.2 Reverse Phenomics

Reverse phenomics attempts to find out why the germplasm is behaving better (Furbank and Tester, 2011).The phenotype or desired trait such as drought tolerance present in a particular germplasm – is already known.Researchers then try to find out the mechanisms that control the trait and the gene(s) underlying the mechanism (Furbank and Tester, 2011). This is done by large- scale physiological and biochemical analysis and then linking the data with genes participating in particular biochemical or physiological pathway. Once the candidate gene(s) has been identified by reverse phenomics approach, then expression pattern of the candidate gene(s) will be compared with other genotypes. Thus, reverse phenomics is the detailed dissection of mechanisms underlying specific traits which allows exploitation of this mechanism or the candidate gene(s) associated with the trait which can be introgressed into new varieties or can be transferred to other plant species using genetic transformation technology (Furbank and Tester, 2011).

III. PHENOTYPING

Phenotyping is the measurement of aspects of plant growth, development, and physiology and arises from interactions between genotypes and environment, including photosynthetic efficiency, rates of growth, disease resistance, abiotic stress tolerance, gross morphology, phenology, and, ultimately, yield components and yield (Hickey *et al.,* 2019). Plant breeding and biotechnology promote the development of new cultivars for sustainable agriculture. In order to enhance the selection process, robust phenotyping is critical because it is a basic tool to determine line selection at each stage of the years-long breeding pipeline. Traditional plant phenotyping for breeders includes walking through their trial fields and scoring plots based on how they look, taste, and/or feel. Improvements in phenotyping methods are highly desired and must address the balance of accuracy, speed, and cost. Engineered phenotyping can augment what the breeders can see and offer better phenotype-based choices.

3.1 High Throughput Phenotyping (HTP)

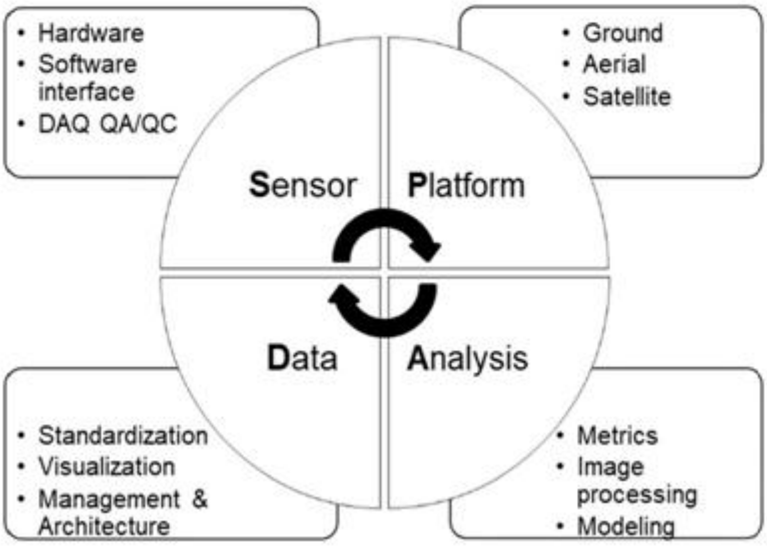
High throughput phenotyping (HTP) platforms could provide the keys to connecting the genotype to phenotype by both increasing the capacity and precision and reducing the time to evaluate huge plant populations. To replace the laborious and inconsistent method of conventional manual phenotyping, breeding industry and public institutes are highly motivated to deploy image-based automated high throughput phenotyping (HTP). However, it is challenging to achieve a reliable imaging solution due to the variability of images affected by lighting intensity and angle. To get to the point of predicting the real-world performance of plants, HTP platforms must innovate and advance to the level of quantitatively assessing millions of plant phenotypes. To contribute to this piece of the challenge, a semi-automated HTP analysis pipeline using a low cost unmanned aerial system (UAS) platform, which will increase the capacity of breeders to assess large numbers of lines in field trials. High-throughput phenotyping, particularly through the application of remote sensing tools, offers a rapid and non-destructive approach to plant screening (White *et al.,* 2012). Recent advances in remote sensing technologies as well as in data processing has increased applications in both field and controlled growing conditions (Araus and Cairns, 2014) with important consequences for crop improvement.

A plant phenotype is a set of structural, morphological, physiological, and performance- related traits of a given genotype in a defined environment (Granier *et al.,* 2014). The phenotype results from the interactions between a plant's genes and environmental (abiotic and biotic) factors. Plant phenotyping involves a wide range of plant measurements such as growth development, canopy architecture, physiology, disease and pest response and yield. In this context, HTP is an assessment of plant phenotypes on a scale and with a level of speed and precision not attainable with traditional methods (Dhondt *et al.,* 2013), many of which include visual scoring and manual measurements. To be useful to breeding programs, HTP methods must be amenable to plot sizes, experimental designs and field conditions in these programs. This entails evaluating a large number of lines within a short time span, methods that are lower cost and less labor intensive than current techniques, and accurately assessing and making selections in large populations consisting of thousands to tens-of-thousands of plots. To rapidly characterize the growth responses of genetically different plants in the field and relate these responses to individual genes, use of information technologies such as proximal or remote sensing and efficient computational tools are necessary.

The increase of interest inground-based and aerial HTP platforms, particularly for applications in breeding and germplasm evaluation activities (Furbank *et al.,* 2011). Ground-based phenotyping platforms includemodified vehicles deploying proximal sensing sensors (Busemeyer *et al.,* 2013). Measurements made at a short distance with tractors and hand-held sensors that do not necessarily involve measurements of reflected radiation, are classified as proximal sensing. Proximal, or close-range sensing, is expected to provide higher resolution for phenotyping studies as well as allowing collection of data with multiple view-angles, illumination control and known distance from the plants to the sensors (White *et al.,* 2012). The ground-based platforms do have limitations mainly on the scale at which they can be used, limitations on portability and time required to make the measurements in different field locations. As a complement to ground-based platforms, aerialbased phenotyping platforms enable the rapid characterization of many plots, overcoming one of the limitations associated with ground-based phenotyping platforms. There is a growing body of literature showing how these approaches in remote and proximal sensing enhance the precision and accuracy of automated high-throughput fieldbased phenotyping techniques (Berger *et al.*, 2010). One of the emerging technologies in aerial based platforms is UAS, which have undergone a remarkable development in recent years and are now powerful sensor-bearing platforms for various agricultural and environmental applications (Baluja *et al.*, 2012). UAS can cover an entire experiment in a very short time, giving a rapid assessment of all of the plots while minimizing the effect of environmental conditions that change rapidly such as wind speed, cloud cover, and solar radiation. UAS enables measuring with high spatial and temporal resolution capable of generating useful information for plant breeding programs. Different types of imaging systems for remote sensing of crops are being used on UAS platforms. Some of the cameras used are RGB, multispectral, hyper-spectral, thermal cameras, and low-cost consumer grade cameras modified to capture near infrared (NIR) (Chapman *et al.*, 2012). Consumer grade digital cameras are widely used as the sensor of choice due to their low cost, small size and weight, low power requirements, and their potential to store thousands of images. The consumer grade cameras often have the challenge of not being radio-metrically calibrated. Radiometric calibration accounts for both variations from photos within an observation day along with changes between different dates of image. The result of radiometric calibration is a more generalized and, most importantly, repeatable, method for different image processing techniques (such as derivation of VIs, change detection, and crop growth mapping) applied to the orthomosaic image instead of each individual image in a dataset. There are well-established radiometric calibration approaches for satellite imagery. These approaches are not necessarily applicable in UAS workflows due to several factors such as conditions of data acquisition during the exact time of image capture using these platforms. There are many private and public sectors pursuing plant phenotyping, with the goal of developing and implementing new HTP approaches that accelerate plant breeding for food, fiber, and bioenergy crops in certain environments.

3.2 Components of High Throughput Phenotyping

The HTP system consists of four components including the sensor, platform, analysis, and data, so called SPAD. These four components are interrelated and technically connected for seamless integration.



Components of high throughput phenotyping (Kim *et al.*, 2019)

Sensor: The sensor is the first component to consider when answering questions of what plant traits are of interest and what phenotypic metrics can be delivered. Typical phenotypic metrics include plant vigor, biomass, canopy temperature, plant height, stand count, etc. There are several types of sensors that can be used for phenotyping: multispectral, hyperspectral, thermal, and light detection and ranging (LiDAR). Spectral and morphological features are commonly measured by multispectral cameras that can estimate vegetation indexes, a leaf area index, stand count, yield, and growth rate. Plant height can be measured by LiDAR (Bai *et al.*, 2019).

Canopy temperature can be measured by IR thermometer (Thompson *et al.* 2018) thermal imager (Bai *et al.*, 2019) and has been commonly used for plant stress detection and phenotyping. Other metrics such as CO2and chlorophyll are also measured by a gas analyzer and chlorophyll sensors, respectively. Among the variety of choices of sensors and metrics, the common consideration is to find cost-effective and robust sensors.

HTP system developers need to take a comprehensive decision for sensor selection by leveraging the sensor performance and operational cost. The sensor selection also must be coordinated with the platform selection to fit its size, weight, mounting, and data rate on the platform in height and speed. Multispectral cameras capture a spectral image and can deliver both spectral and morphological features through image analysis.

Platform: In HTP system equipped with various sensors can be mounted on three different HTP platforms. They are ground, aerial, and satellite. Each platform has its own pros and cons, and the developers need to know what outcomes and limitations are expected from each platform. Although there are still positive aspects on each platform, in general, the HTP research trend is shifting from the ground to the aerial/drone platform with several reasons such as increased coverage and mobility due to shortened acquisition window that minimizes the solar radiation effects. The future HTP platform design must accommodate the two most important factors: the field coverage in acquisition and turnaround time in data analytics.

Ground Platform: The ground platform is the most commonly used for plant phenotyping under two different environmental setting i.e., indoors and outdoors. The most commonly used HTP platform is an outdoor ground platform operated by a cart or a tractor. Manual push-behind carts (Crain *et al.,* 2016). A motorized cart can be equipped with chain-driven electric motors and needs gear reduction to increase torque enough to smoothly start from a stop. Built-in geared hub motors are another option to simplify the drive and gear design and avoid chain maintenance. The motorized cart is controlled by a remoter controller through PWM signals and can be programed with GPS readings and path planning for upgrading to autonomous navigation. Tractor-based ground platforms are also widely studied (Wang *et al.,* 2019). These ground platforms can measure great details of plant morphological and spectral features using proximal and spectral sensors. There are two types of indoor HTP platforms depending on stationary or mobile plants. If plants are fed into a scanning chamber on a conveyer belt, all sensors are mounted in the sensor package in different angles. The mobile plant-based HTP platform takes advantage of detailed observation under the controlled environment using various sensors such as VIS, NIR, fluorescence, stereo cameras, thermometer, and LiDAR. The disadvantage of this method is a long operational time to feed all plants to the scanning chamber (e.g., 30 h for 30,000 images of 1140 plants (Fahlgren *et al.,* 2015) and frequently occurring maintenance issues in software with the sensors and hardware with the conveyer machine. The stationary plant-based HTP platform requires mobile sensors in a gantry mode to move to a target plant (Burnette *et al.,* 2018) using commercially available devices. The sensors can also be stationary using a grid of sensors permanently mounted over the entire area of the target plants, which relieves the operational issues with time delay and allows a localized troubleshooting of an individual sensor instead of the entire system stop caused by a single issue of the moving machine or sensor on the mobile plant-based platform.

- **Aerial Platform:** Aerial phenotyping has two platforms: a manned aerial vehicle (MAV) and an unmanned aerial vehicle (UAV). MAV has been well established in urban and forest industry and increasingly adopted for agricultural applications (Yang *et al.,* 2013). Sensors are mounted on a small airplane, popularly on Piper Saratoga. UAV has been broadly adopted for agricultural applications: crop water use (Thorp *et al.*, 2018), cotton boll detection (Yeom *et al.,* 2018), maize green leaf index (Blancon*et al.*, 2019), bio-enthaol in cereals (Ostos-Garrido*et al.,* 2019), and plant phenotyping (Sagan *et al.,* 2019). UAV platform is capable of carrying additional sensors of LiDAR, thermal, and/or hyper-spectral sensors and expected to become more popular with decreasing costs and improvements in quality.

- **Satellite Platform:** A satellite platform has been used by several government agencies to estimate weather conditions, crop areas, and yield. Private firms are investing in satellite remote sensing technology for decision support on crop production and developing models from high throughput satellite imagery for crop health monitoring. As satellite and sensing technology grows, however, it is still possible for satellite as a future HTP platform when those limiting factors are addressed.

IV. ANALYSIS OF DATA

HTP has high potential to improve genetic modeling and expedite the identification of germplasm that increases the yield and productivity of crop plants. Several analytic toolboxes have been published: PlantCV (Fahlgren *et al.*, 2015), Lemna Grid (Honsdorf *et al.,* 2014), HTPheno (Hartmann *et al.*, 2011), and integrated analysis platform (Klukas *et al.,* 2014). Lack of standardized analytic tool delays data processing from different platforms and sensors and is a major hurdle in the HTP processing pipeline. Despite different sensors and platforms in existence, image analysis and trait extraction are common challenges for image-based phenotyping platforms, and thus open-source trait extraction software with a mechanism for community development will help to alleviate the phenotyping bottleneck on crop improvement (Fahlgren *et al.,* 2015). The main aspects for HTP image analytics include image formation, radiometric calibration, stitching, and image analysis.

HTP data management is increasingly important as the big data are built up for spatial and temporal phenotypes from multiple fields in various regions under different weather conditions. HTP study generates huge volume of raw data in diverse formats and undergo data abundance with high-resolution, redundancy, and invalid or unnecessary data, and thus HTP data are heavily involved in big data management. Collecting phenotypic data by automated HTP machine like TERRA-REF, the world's largest robotic field scanner, can produce up to 10 TB data per day (Binder, 2018). The metadata are essential to integrate the heterogeneous HTP data, but generating metadata for geospatial data is challenging due to the data's intrinsic characteristics of high-dimensionality and complexity such as space-time correction and dependency (Yang *et al.,* 2017).

Phenotypic data collected in the field are transferred to cloud database (DB) through a local storage, accessed via cloud for data processing and analysis using an analytic toolbox, and cataloged for visualization once data is processed, visualization is implemented to allow the end- users to access field images and understand the results. HTP research has a challenge to close a gap between the plant science and data analytics to ensure for plant science communities know how to use the HTP data and for data analytics communities understand how to deliver actionable results for scientists and farmers. One of the concerns in big data driven agricultural community is lack of data quality. There is a consensus that "garbage in" in terms of primary data quality results in "garbage out" of final data quality (Shakoor *et al.,* 2019). For instance, poor quality of the positive images used for machine learning models would mislead to a poor quality of prediction results. Quality assurance and check become more important for sensor precision and consistency, and quality protocols need to be developed and standardized for the future HTP research programs.

V. A NEW TOOL FOR ADVANCEMENT OF TRADITIONAL CROP IMPROVEMENT

Crop Breeding started with the selection of plants based on phenotype. Advances in molecular biology led to the development of marker assisted breeding, Genome Wide Association Studies, Genomic selection where a large population is to be assessed for effective selection. Improvements in phenotyping are likely to be necessary to take advantage of breakthroughs in conventional, molecular, and transgenic breeding and ensure genetic improvement of crops for future food security (Arausand Cairns, 2014). For field phenotyping, there are a number of platforms that are used with various sensors, including multispectral, hyperspectral, IR and RGB cameras. They range from simple ground-based platforms i.e., monopods and tripods, to complex unmanned ground vehicles and unmanned aerial vehicles (UAVs). Use of High throughput phenotyping could contribute significantly to the fastening of the breeding programme.Currentadvanced techniques including thermal, near-infraredsensing, fluorescence imaging, 3D scanning, RGB imaging, multispectral and hyperspectral sensing are lucrativelyused for plant growth and development identification, quantification and monitoring, disease monitoring, and abioticstress tolerance (Furbank *et al.,* 2019). RGB imaging and along with multi sensor and portable spectrometers help in the phenotyping of plants and helps in monitoring the stem water potential, leaf conductance and leaf area index. HTP based field screening is used for measuring canopy temperature under heat and cold stress and is strongly associated with the deep root system and transpiration flux. This also could be analysed using infrared thermography, which analysesleaf surface temperatureto study stomatal conductance and plant–water relations.Chlorophyll fluorescence imaging captures the changes in photosyntheticperformance of plants related to salt stress. The colour of the images reveals the difference between the normal and the affected plants and helps in the selection and development of tolerant plants. These imaging techniques also helps in the identification of pathogen and insect infestations in plants.Thus, high-throughput screening could providehighly accurate and time-lapsed inspection to monitorplant diseases.Plant breeders are under tremendous pressure to create high yielding cultivars due to the growing populationand HTP can support these efforts by supplementing mass-scale germplasm screening.Multiple sensors, ease handling, and improved mobile networks are innovative elements that would be needed for portable HTP. HTP in crop breeding may be revolutionised by such sturdy gadgets (next generation cellphones), cloud computing, and usage of Artificial Intelligence (AI). Another goal will be to concentrate on management techniques for breeding data and networking for mutual benefit (Jangra *et al.,* 2021).Aerial sensing technologies offer radically new perspectives for assessing these traits at low cost, faster, and in a more objective manner. Makanza *et al.,* 2018 used the UAV equipped with RGB camera to analyze the crop cover and canopy senescence in maize field. They concluded that UAV-based aerial sensing platforms have great potential for monitoring the dynamics of crop canopy characteristics like crop vigor through ground canopy cover and canopy senescence in breeding trial. Increased precision and accuracy with less time and expense for data collection, increases the efficiency of selection.

VI. BIOTIC STRESS RESISTANCE ENHANCEMENT THROUGH HIGH THROUGHPUT PHENOTYPING

For the intent of identifying and evaluating both simple and complex plant traits for crop improvement, such as plant height, biomass, flowering time, and grain yield, high throughputphenotyping has recently emerged as a rapidly evolving discipline by flourishingly integrating disciplines of plant science, engineering and computational science (Tanger *et al.*, 2017). Field crop breeding may be hastened up, the rate of genetic gain and disease tolerance increased, and entire sets of field data can be produced using phenotyping methods that boost plant screening (Mahlein, 2016). HTP makes it possible to assess characteristics including seedling vigour, flower counts, biomass and grain production, height, leaf erectness, and canopy structure in a precise, automated, and repeatable manner (Shakoor *et al.,* 2017).Moreover, HTP could potentially be used to test physiological characteristics including photosynthesis, transpiration, pest infestation, pathogen incidence, and stress tolerance. To minimize yield losses due to plant diseases, credible and timely identification biotic stress is essential. Conventionally plant disease diagnosis depends on visual evaluation and symptom detection which is tiring and error-some. In recent past, plant diseases have been identified, measured, and monitored with efficacy using HTP.Techniques and recent advancements in RGB imaging, 3D scanning, thermal and near-infrared sensing, multi-spectral and hyperspectral sensing, and fluorescence imaging has enhanced the overall efficiency of HTP in biotic stress identification also.

The scales of HTP platforms for biotic stress management vary from single plant organ/plant to full field, from ground vehicles to UAVs and satellites (Liebisch *et al.,* 2015). In particular, satellite platforms have high potential for identifying and tracking crop diseases over large cultivated lands. As of late, satellite platforms with multi- and hyperspectral sensors, such as NASA's Ecosystem Spaceborne Thermal Radiometer Experiment on Space Station (ECOSTRESS), Soil Moisture Active Passive (SMAP), and HyperspectralInfraRed Imager (HyspIRI), have been developed with the goal of gathering high-resolution spectral and environmental data to help develop new approaches for drought mitigation and water use efficiency (Lee *et al.,* 2015, Entekhabi *et al.,* 2010, Fisher *et al.,* 2015).

Laterly, various sensor technologies has been advocated for different plant pathogen systems (Mahlein, 2016) viz. RGB/ Stereo RGB, 3D laser scanner, multispectral sensor, thermal infrared (IR), near infrared (NIR), visual near IR (VNIR), shortwave IR and fluorescence sensors. Each sensor has its own potential application in assessment of different disease/pathogens in different crop fields. RGB cameras with their high-resolution data and fast acquisition rates allows for assessment of plant growth dynamics, plant and root architecture and disease screening (Smith, 2009 & Suguria *et al.,* 2016). The 3D lasers have been in use for more than 10 years in HTP due to their resolution and precise scanning. They are used in the assessment of leaf area index, canopy structure and height (Roscher et al, 2016). A thermal IR sensor identifies the temperature fluctuations in the crop canopy level which greatly aids in the identification of disease outbreak and water stress condition. The visible (400-700 nm), near infrared (700-1300 nm), and short-wavelength infrared (1400-3000 nm) wavelength bands of electromagnetic energy reflected by plants are all captured by multi- and hyperspectral imaging methods which convey information about leaf physiology of the crop including leaf pigments and leaf contents (Yendrek *et al.,* 2016). Using hyperspectral imaging the severity of disease can be estimated (Kuska *et al.,* 2015). For instance, levels of stripe rust infection and N deficit in wheat crops have been measured using successive combinations of several hyperspectral indices (Devadas *et al.,* 2015). Fluorescence imaging measures the light that the chlorophyll a emits back into the atmosphere and provides insights into the photosynthetic system under biotic and abiotic stress. Disturbances in fluorescence measurements can frequently occur before the onset of apparent symptoms, making them useful for early diagnosis (Konaz *et al.,* 2014, Tatagiba *et al.,* 2015). Fluorescencespectros copy, for instance, was used to identify citrus trees with bacterial infections causing citrus greening (Wetterich *et al.,* 2017).

Once the initial data is obtained, it is used for data preparation using open-source platforms such as Python and ImageJ to analyse the yield attributes and disease diagnosis (Schindelin *et al.,* 2012, Van der walt *et al.,* 2014). From the unique data, novel patterns, forecast trends are drawn using probabilistic algorithms. This process is called machine learning (ML). Even though there are numerous ML techniques have been used for identification and classification for phenotyping, each technique has their own shortcomings. So it is important to wisely select the ML methods. However, as picture databases grow in size, scope, and complexity, ML continues to stand as the best practical method for deriving useful insights and analysis from field crop image datasets, which are exponentially expanding (Shakoor *et al.,* 2017).

VII. ROLE IN GENOMICS

Agriculture research has been transformed by plant genetics and genomics, and crop plants have amassed a tremendous number of genomics resources. A deeper comprehension of the physiology and genetic underpinnings of critical traits related to yield, quality, and biotic and abiotic stresses is required for the effective exploitation of the germplasm using genomics for crop development (Edwards *et al.,* 2012). Therefore, in order to take full advantage from this plethora of genomic data for crop development, it is necessary to link and incorporate it with the phenotypic data in a real-life scenario (Furbank and Tester, 2011). As a result, precise and economical phenotyping is crucial. The area of phenomics has seen significant advancements that will allow for a more accurate and thorough screening of the features. These developments have aided in the creation of high throughput phenotyping systems for germplasm screening. Utilizing techniques/approaches enhanced the gathering of phenotypic data more precisely and economically with less experimental noise. For this reason, proper phenotyping will be helpful in estimating the true heritability of a trait, which is necessary to create genetic improvement through genomic selection (Mir *et al.,* 2015; Kumar *et al.,* 2020). As a consequence, genetic resources may be used to their fullest extent by locating the true QTL for complicated characteristics, discovering the genes in the QTL, and determining the function of those gene sequences whose functions are yet unknown (Cobb *et al.,* 2013). Plant breeders will shortly have access to phenotyping facilities that will be routinely used to screen huge populations for genetic improvement in crops and precise gene modification using genomics.

VIII. CONCLUSION

Machine Learning tools provide a very powerful framework to assimilate data, and the utility of these tools is especially important considering current progress in HTP approaches that easily generate of data. Appropriate choice and usage of ML tools is crucial for obtaining the maximum possible benefits of these sophisticated approaches. Efficient, high-throughput phenotyping methods can be implemented only when data accuracy, process speed, and cost is well balanced within the permitted limits. It is clear that limitations of the technology exist, such as low payload and a narrow area for image collection. The utilization of cost-effective commercial UAV platforms for phenotyping and methodologies for high-throughput phenotyping accelerate plant breeding cycles. To promote the next green revolution in crop breeding, the development of an International Crop Phenome Project (ICPP) should also be encouraged.

Questions

1. Differentiate between conventional phenotyping and High Throughput Phenotyping
2. Detail the components of High Throughput Phenotyping
3. Write in detail about the shortcomings of implementation of High Throughput Phenotyping
4. Role of High Throughput Phenotyping in genomics
5. Role of High Throughput Phenotyping in biotic stress breeding

Self Assesment

2. Some examples of high-throughput phenotyping tools
   a. Genomics, transcriptomics, and metabolomics
   b. Forward phenomics and reverse phenomics
   c. Sensors, imaging systems, and computing power
   d. Climate change, resource depletion, and population growth

3. Forward phenomics work by
   a. Analyzing the core molecular genetics of plants
   b. Studying the effects of climate change on crop performance
   c. Identifying the mechanisms underlying specific traits
   d. Using automated imaging technology to screen germplasm for valuable traits

4. What is phenomics?
   a. Study of climate change effects on plant genetics
   b. Acquisition of multidimensional phenotypic data in an organism as a whole
   c. Use of automated imaging technology to screen germplasm for valuable traits
   d. Analysis of the core molecular genetics of plants

5. What is reverse phenomics?
   a. The study of climate change effects on plant genetics
   b. Identifying valuable traits in germplasm using automated imaging
   c. Studying the core molecular genetics of plants
   d. Dissecting mechanisms underlying specific traits and identifying associated genes

6. Phenomics affect agriculture by
   a. Analyzing the core molecular genetics of plants
   b. Studying the effects of climate change on crop performance
   c. Providing large-scale, high-quality trait data through advanced technologies
   d. Manually phenotyping plants for accurate data collection

7. What is the primary goal of Forward Phenomics?
   a. Analyzing core molecular genetics
   b. Identifying valuable traits in germplasm
   c. Studying the effects of climate change on crops
   d. Dissecting mechanisms underlying specific traits

8. After screening, what is the next step for plants with target traits in Forward Phenomics?
   a. Discarding them for further analysis
   b. Growing them to produce seeds
   c. Analyzing their core molecular genetics
   d. Studying their physiological and biochemical behavior

9. Phenotyping impact crop breeding by
   a. Analyzing the core molecular genetics of plants
   b. Manually phenotyping plants for accurate data collection
   c. Providing large-scale, high-quality trait data through advanced technologies
   d. Studying the effects of climate change on crop performance

10. Why is robust phenotyping considered critical in plant breeding?
   a. To analyze core molecular genetics
   b. To expedite the identification of germplasm
   c. To determine line selection at each breeding stage
   d. To replace traditional manual phenotyping methods

11. What is the significance of barcodes in the Forward Phenomics process?
   a. Identifying climate change effects on plants
   b. Manual data collection for large populations
   c. Labeling plants for automated screening
   d. Studying the core molecular genetics of plants

12. Phenotyping is primarily concerned with
   a. Core molecular genetics
   b. Plant growth, development, and physiology
   c. Climate change effects on crops
   d. Soil composition and nutrient levels

13. High Throughput Phenotyping (HTP) aims to achieve
   a. Decreasing the capacity and precision of evaluations
   b. Increasing the time required to evaluate plant populations
   c. Providing keys to connect genotype to phenotype
   d. Using manual phenotyping for large plant populations

14. Challenge associated with image-based automated high throughput phenotyping (HTP)
    a. **Consistency of lighting intensity and angle in images**
    b. Dependence on traditional manual phenotyping methods
    c. Lack of interest from the breeding industry
    d. Inability to assess plant phenotypes accurately

15. What is one of the advantages of aerial-based phenotyping platforms?
    a. Limited coverage and mobility
    b. **Increased scale of their usage**
    c. Dependence on ground-based platforms
    d. Longer operational time for data analytics

16. The main challenge involved in HTP data management
    a. Lack of standardized analytic tools
    b. Consistency of lighting in images
    c. **Generating metadata for geospatial data**
    d. Inability to collect phenotypic data automatically

17. RGB imaging spectrometers is used for phenotyping the following data
    a. Stem water potential
    b. Leaf conductance
    c. Leaf Area Index
    d. **All of these**

18. Leaf surface temperature fluctuations are diagnosed using
    a. RGB imaging
    b. **InfraRed Thermography**
    c. Fluorescence spectroscopy
    d. Hyperspectral sensing

19. Photosynthetic parameters are measured using imaging
    a. RGB imaging
    b. InfraRed Thermography
    c. **Fluorescence spectroscopy**
    d. Hyperspectral sensing

20. Biotic stress breeding uses the imaging for diagnosis
    a. RGB imaging
    b. InfraRed Thermography
    c. **Fluorescence spectroscopy**
    d. Hyperspectral sensing

21. Wavelength of visible spectrum is
    a. 600-700 nm
    b. 400-700 nm
    c. 300-500 nm
    d. 1400-1500 nm

22. From the unique data, novel patterns, forecast trends are drawn using probabilistic algorithms which is called .
    a. Data processing
    b. Machine learning
    c. Phenomics
    d. Data storage

23. Dynamics in Crop canopy can be supervised using
    a. RGB imaging
    b. InfraRed Thermography
    c. UAV Aerial sensing platform
    d. Fluorescence imaging

24. Allows for assessment of plant growth dynamics, plant and root architecture and disease screening
    a. InfraRed Thermography
    b. RGB imaging
    c. Fluorescence spectroscopy
    d. Hyperspectral sensing

25. Wavelength of Near infrared radiation
    b. 600-700 nm
    c. 400-700 nm
    d. 700- 1300 nm
    e. 1400-1500 nm

26. Wavelength of Short-wavelength infrared
    b. 1400-3000 nm
    c. 600-700 nm
    d. 400-700 nm
    e. 700- 1300 nm

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