Review Article

Phenotyping in Plant Breeding using Modern Tools:A Review

Abstract

The need for food globally is rising quickly. According to assessments, global food output must double to meet anticipated population growth projections by 2050. The ability to precisely phenotype crop plants in the field has been made possible by contemporary high-throughput plant phenotyping systems (HTPPs).This has been made possible by developments in automation, robotics, precise environmental control, and remote sensing technologies. We are discussing some phenotyping tools phenopsis, phenomics, phenoscope, HPGA, The Plant Accelerator, Biotron, LEPSE, LemnaTec etc. This paper examines many phenotyping contexts, such as breeding, genetic resource discovery, and translational research to provide more breeding resources, and how the various phenotyping categories mentioned above relate to each of these situations. By bridging the gap between genotype and phenotype and improving the effectiveness of selection for maximizing the genetic gain, several HTP tools and platforms have been created globally to enable breeding operations reach their full potential.

Keywords- Automation, Phenopsis, Robotics, LemnaTec

Introduction

To evaluate plant attributes (such as growth, morphology, architecture, function, and composition) at various scales of organization, a new discipline called plant phenotyping has been developed [6]. Wilhelm Johannsen, a Danish plant scientist, is credited for coining the words "phenotype" and "genotype" [24]. Plant phenotyping is a crucial technique for addressing and comprehending plant-environment interactions and their application in crop management practises, biostimulant effects, microbial communities, etc. [41,35, 34]. One of the main phenotyping applications in use today is plant breeding, and breeding aims influence many improvements in plant phenotyping [11]. In this situation, emerging methods must offer benefits in terms of throughput, field usability (across a wide range of weather conditions), and, in particular, the utility of the breeding process itself and the heritability of the determined characteristics[45]. The features of an organism that are the consequence of interactions between genetics, environment, and crop management are known as phenotypes. To characterise the phenotypic space produced by a certain genome or group of genomes in more detail, phenomics entails accumulating relevant phenotypic data at various organisational levels [7]. Consequently, plant phenotyping may function in a variety of contexts, from controlled to field circumstances, and at various degrees of resolution and dimensionality, from the molecule to the entire plant. The ultimate objective is to integrate knowledge from the bottom up to generate cultivars with improved performance, even while each level concentrates on certain features. In this sense, using plant phenotyping techniques in breeding programmes has developed into a potent research tool to assist breeders in creating cultivars more adaptive to many difficult environmental circumstances [5]. The

phenotype, which was created by an organism's interactions with its environment from the very beginning of its existence to the conclusion, presents various extra difficulties for the quantitative analysis [20]. Recording biological systems and their environments in the many spatial and temporal dimensions required to comprehend the genesis of a particular phenotype has long been a nearly difficult undertaking. In contrast to, for example, medicine, which "only" analyses a single species with extremely limited variety in compared to, for example, plant species, biology deals with numerous species that have quite various habits and designs. Furthermore, plants in particular have extremely malleable phenotypes, which aid in their environment-specific adaptability. For instance, plants exposed to a restricting environment (such as a drought) stay (usually) smaller and often change their structure (shoot and root) and physiology[43]. This flexibility of the phenotypic even creates feedback loops. They become more resistant to the following restriction as a result. As a result, the flexibility of the phenotypic in response to the environment frequently has an impact on how exposed the plant is to the environment later in its life. Phenotypes are highly dependent on earlier encounters with the crop and their adaptation to it as a result of this "memory effect" [38]. When environmental signals that influenced mother plants during seed filling create maternal or epigenetic impacts on the seed composition and quality, this also includes effects that traverse generations [26].

Conventional Phenotyping

Traditional phenotyping has several bottlenecks that are used to justify the tests, which slow down the research programme and are time- and labor-intensive. The entire phenotyping of the bigger plot is highly challenging, and even if completed, may not be accurate. Traditional phenotyping involves visual screening, which is useless for identifying physiological and biochemical traits. Destructive test is part of the biochemical characterisation process. Because traditional phenotyping is prone to inaccuracy, it is challenging to justify the experiment precisely. This is the driving force behind the development of the phenotyping science [25].

High Throughput Phenotyping

Platforms for high-throughput phenotyping combine a platform for data processing with data gathering hardware and a control terminal. In order to quickly assess plant growth activities and physiological health, they primarily collect phenotypic data using non-invasive imaging and spectroscopic methods. High-throughput phenotyping, as opposed to conventional phenotyping techniques, enables the dynamic monitoring of plants at various growth stages as well as the simultaneous data collecting for many attributes in huge populations. Second, trait characterization based on spectra or pictures is more objective than more conventional techniques like visual scoring, which are subject to subjective interpretation. Thirdly, it enables non-destructive determination of biochemical parameters based on modelling, eliminating the need for time-consuming procedures. High-throughput phenotyping methods have been developed during the past few years for a variety of targets, including cells, seeds, shoots, leaves, roots, individual plants, and canopies [44, 36]. High-resolution imaging techniques, such as micro-computed tomography (micro-CT) imaging and microscopic imaging, could be used to analyse the number of cells, changes in cell structure, growth rate of cells, and tissue morphology in terms of cells and tissues [9, 12]. When it comes to phenotyping seeds, visible light imaging has been extensively used to characterise

morphological features such colour [3], coleoptile length, and germination rate [28]. A portable device based on a smartphone has been developed for the aim of phenotyping seed morphological features [46]. Additionally, utilising X-ray imaging, seed morphometric traits and tissue integrity have been assessed [31]. By determining the amounts of protein, oil content, and fatty acids in seeds, near-infrared spectroscopy and time-domain pulsed nuclear magnetic resonance (NMR) have demonstrated advantages in analysing the biochemical components [23, 29]. For organs, individual plants, and canopy, the characteristics and potential of numerous high-throughput phenotyping techniques for obtaining phenotypes at these scales have been detailed[21].

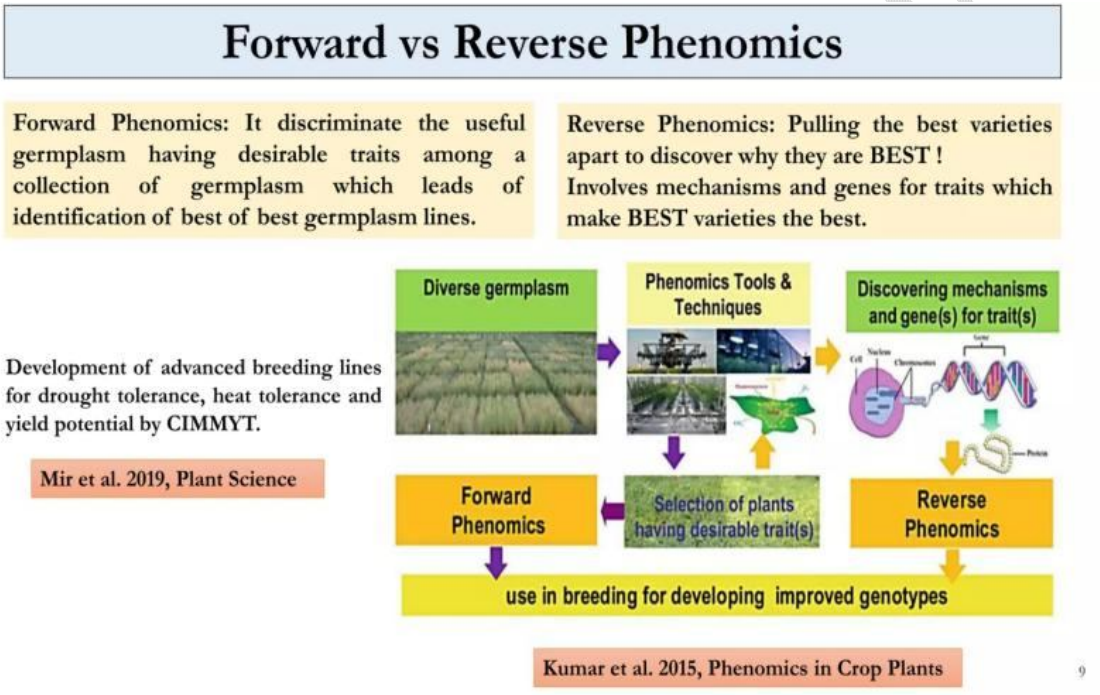

Fig 1: Forward vs Reverse Phenomics [25, 33]

High Throughput Phenotyping Platforms

Precision agriculture has become a key discipline for the most effective and comprehensive use and management of natural resources during the past two decades. Recently, a variety of applications for laboratory research and screening systems for horticulture and production systems have been accessible thanks to automation, robotics, high-speed computers, novel sensors, and imaging technologies [4,16, 10, 47]. As a result, precision phenotyping of plants has evolved and now calls for HTPPs. Over the past fifteen years, a variety of automated plant phenotyping platforms have been created. Although limited to a few specific species, such as Arabidopsis [2, 14, 37,1] cotton, and several of the major cereals [13,18], they are capable of large-scale phenotyping.

The majority of the high-speed, fully automated HTPPs that are now in use across the globe are housed in greenhouses or growth chambers. These platforms evaluate crucial aspects of plant growth and development and analyse their genetics using robotics, precise

environmental management, remote sensing technologies, global positioning systems, and high-speed computer facilities. Large seed firms and cutting-edge agricultural research organisations from all over the world administer the majority of these HTPPs[32].

1.Phenopsis

Phenopsis is an information system created for the storing, viewing, and sharing of offline data gathered by experimenters as well as experimental metadata and online data generated by the PHENOPSIS platform [8]. Granier *et al*. [15] created this technique for repeatable phenotyping of Arabidopsis thaliana. It is a prototype made by Apilogic (Fondettes, France) that is supported by 14 trays on a steel frame. Each tray includes 36 slots that can accommodate pots and an arm made of metal that can move in accordance with a programme created by Apilogic using the APIGRAPH IP software. Sensors for displacement, balancing, cameras, and irrigation tubes can all be mounted on the arm. The experiment's many components, including as the arrangement of the pots on the trays, the frequency and duration of watering regimens, the timing of the photo shoot, etc., may be readily programmed on a computer using the APIGRAPH IP software. With the aid of software, a computer can also regulate the growth chamber's climate. The computer may then be connected to numerous sensors, such as those that detect light, humidity, leaf temperature, and other micrometeorological factors. For the study of genotype X environment interaction effects on many plant processes, systems like this one generate enormous amounts of environmental data, plant pictures, and phenotypic data [8]. But this system needs protocols for managing datasets, extracting them, and sharing them with the scientific community.

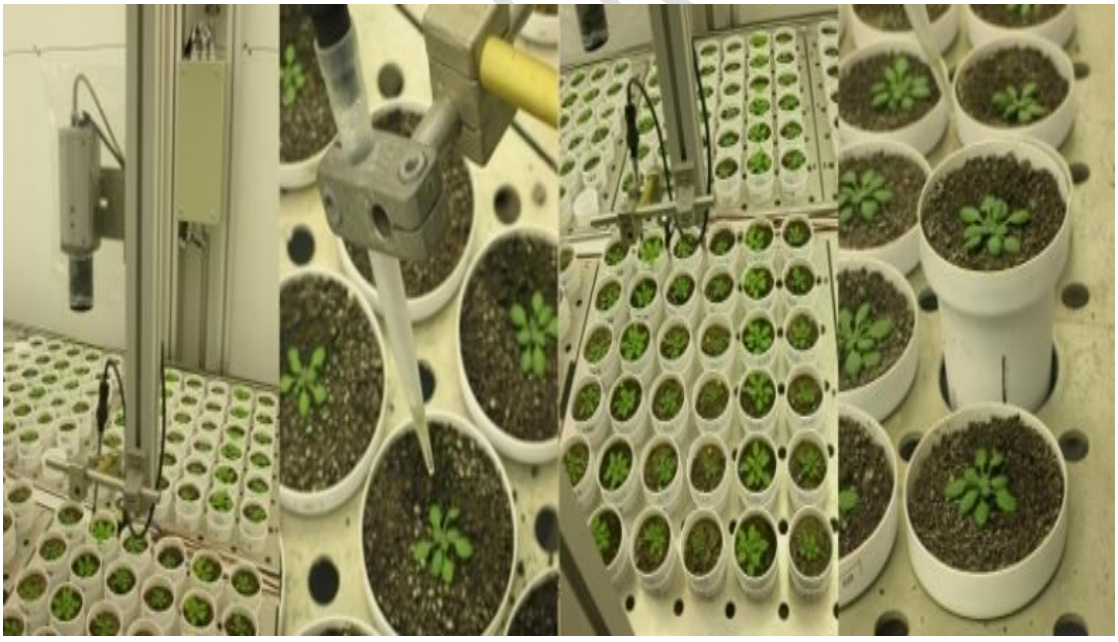

Fig 2 : the PHENOPSIS platform, which enables the nurturing of Arabidopsis plants and the execution of 2D acquisitions of those plants in a controlled environment [14].

2. Phenopsis DB

PHENOPSIS DB offers a solution for the analysis, storage, and sharing of pictures and data gathered in the PHENOPSIS platform using a web interface and web services [14]. For (1)

the visualisation of an experiment's environmental data, (2) the visualisation and statistical analysis of phenotypic data, and (3) the analysis of photographs of the Arabidopsis thaliana plant, this platform offers modules connected to a web interface. The Web interface was created using XHTML, PHP, JavaScript, jQuery, Ajax, and CSS, while the database was created using the MySQL 5.0 Community Server. The database connection was made using the RODBC package in R version 2.9.2. Access to all PHENOPSIS DB metadata is unrestricted. The experiment features and related protocols, genotyping information, a description of the many factors taken into account in an experiment and related procedures, micrometeorological data, and experiment comments are all included in the metadata. PHENOPSIS DB's main benefits and features are its user-friendly Web interface and integration with other databases. In addition to consulting many experiments and genotypes, users of this system may obtain and evaluate publically available datasets, ambient circumstances during an experiment, and various photographs linked to an experiment.

3. Phenoscope

It is a platform with the distinctive ability to automatically regulate watering as 735 different pots are continually rotated around a table. It consists of an aluminium table supported by a steel frame that is kept in a growing chamber. Up to 735 plants may be cultivated and individually phenotyped on this framework. The table has watering and weighing equipment so that the plants may be kept at a certain treatment objective. The table has a closed-circuit track and several pot-holding buttons. The synchronised pusher arms that are used to move the individual pots—each of which is intended to support a single plant—along the guiding rails enable the robot to move each pot sequentially through all conceivable places on the table in a single cycle, up to six times per 24 hours [40]. The way the plants travel is planned such that, despite the environmental variety, each plant encounters the same environmental circumstances on average over time, without any comparative benefit or disadvantage. At the phenotyping station located in the top right corner of the table, non-destructive phenotyping can be carried out. Images of the plants on the Phenoscope are captured by a digital camera, labelled, and stored on an image server for later retrieval and study. Large-scale tests may now be carried out that would not have been feasible or repeatable by hand thanks to the Phenoscope [40].

Fig 3 :Phenoscope: Developed at INRA-IJPB Versailles, this Arabidopsis culture system has the ability to grow and move 735 plants across the culture table, individually modify each plant's water and nutrition status, and track each plant's development using picture analysis [27].

4. HPGA

HPGA is a HTPP for functional analysis and modelling of plant growth. Plant area estimate (PAE) and growth modelling and analysis (GMA) are its two parts. Every plant's area in PAE is calculated from top-view photos in four phases while taking into account the challenging leaf overlap problem. A nonlinear growth model is used in GMA to produce growth curves, which are then the subject of a functional data analysis. A growth curve is produced utilising the observed value of a plant over time in the majority of high-throughput computational phenotyping systems by regularly taking top-view photos [44]. However, leaf overlap, leaf twisting, curling, and circadian motions all impact the apparent value of a plant when viewed from above, leading to erroneous estimations of growth trends. The severity of this issue increases as plants age and develop an excess of bigger, overlapping leaves. In order to more precisely measure plant area, HPGA calculates the leaf overlap percentage. HPGA estimates plant areas accurately through a four-step process that includes plant centre identification, leaf tip identification, leaf area estimation, and plant area measurement [39]. This differs from existing techniques that merely count the number of valid pixels in an image [17,19].For a more accurate assessment of the plant development scenarios, functional data analysis is used to growth curves in HPGA. With this method, you can identify every leaf on a plant from a top-view photograph without having to worry about the leaf segmentation problem. Researchers may automatically produce hundreds or even thousands of observations for each plant using this high-throughput phenotyping approach. The main drawback of this model is that it must be trained afresh with new leaves when switching from one species to another since the leaf-to-area model is genome-specific.

5. The Plant Accelerator

The Plant Accelerator automates high-throughput, non-destructive phenotyping of agricultural plants using digital imaging technology, high-capacity computers, and robotics. It is housed in a specially designed building with the most up-to-date greenhouses, growth chambers, labs and seed storage facilities. The Plant Accelerator, which is situated on the Waite Campus of the University of Adelaide and is funded by the Australian Government through the National Collaborative Research Infrastructure Strategy (NCRIS) and the University of Adelaide, offers users knowledge of plant and soil science. This technique has permitted a variety of research initiatives, including extensive testing of early development, salt tolerance, and water- and fertiliser usage efficiency. The speed and precision of plant physiological measures are increased by using HTPP. Additionally, it facilitates the execution of projects involving sizable plant populations, enabling genetic analyses to be carried out in order to pinpoint the molecular basis of intricate physiological features in addition to fostering a better knowledge of how environmental factors influence plant development and performance. For non-destructive plant phenotyping, the Plant Accelerator employs imaging stations (LemnaTecScanalyzer 3D). Using visible light pictures (RGB), this method can assess shoot area and inferred mass, plant height and breadth, canopy density, other morphometric information, leaf colour, and senescence. Furthermore, programmable watering to plant weight and steady-state fluorescence imaging with blue light broad field excitation (500 nm) enable large-scale research needing regulated watering levels. These techniques also enable assessment of plant senescence, chlorosis, and necrosis[22].

Fig 4 : Photograph of the Plant Accelerator [30]

6. Biotron, Canada

At the University of Western Ontario in Canada, there is a multidisciplinary, experimental climate change research centre called The Biotron. It is a distinctive, specially designed facility with specialised environmental chambers, laboratories, and tools. Earth sciences, microbiology, plants (including transgenics), algae and cyanobacteria, plants (including transgenics), and insects all have different study modules at Biotron facilities. These modules contain labs and equipment that offer specially created controlled conditions and analytical instruments. The majority of Biotron's equipment may be operated or monitored online from any place, and remote users are assisted by technicians and experts on staff at the Biotron. The Biotron is outfitted with a Flow Cytometry Suite, an Analytical Laboratory Suite, and an Imaging and Data Analysis Suite, together with the related imaging and analytical labs. The

Biotron makes it possible to combine environmental and climate change experiments at scales ranging from the molecular to the mini-ecosystem. A secure, remote-accessible central server is networked with several equipment, growth chambers, and imaging devices to provide worldwide access.

Fig 5: Photograph of the Experimental Climate Change Research Centre (University of Western Ontario, Canada.)

7. LEPSE (Ecophysiology Laboratory of Plants Under Environmental Stress), Montpellier

This state-of-the-art phenotyping laboratory, which is situated in Montpellier, can do high-throughput analyses of micrometeorological variables such as soil water content, apex temperature, and others, as well as transpiration, leaf expansion, reproductive development, and canopy architecture. In this facility, further in situ measurements of cell turgor, hormones, and the kinematic study of the rate of cell division and tissue growth are also feasible. Through the use of several plants in various experimental scenarios, the phenotype is addressed through time courses of organ enlargement, plant transpiration, and 3D development of leaf surfaces.

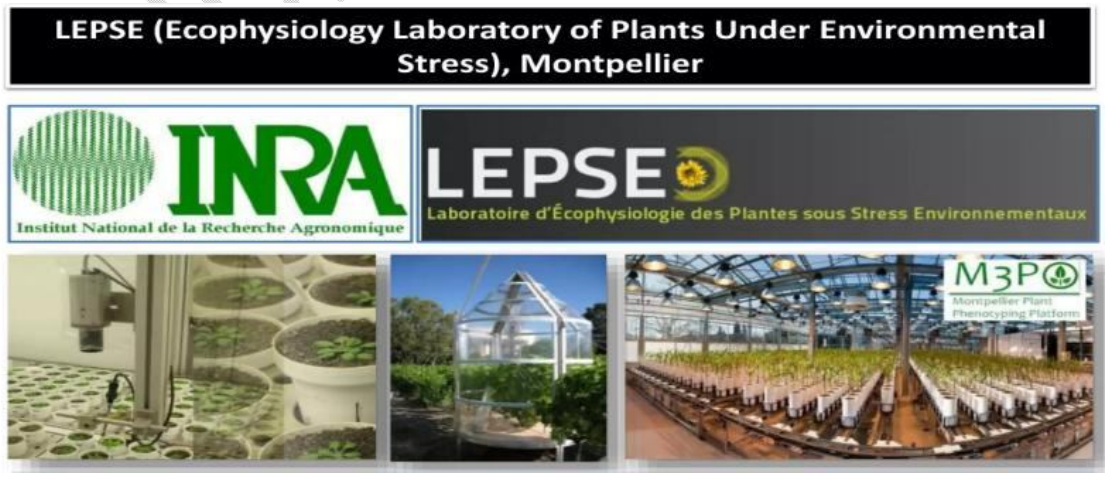

Fig 6:PhenoFab, Wageningen (KeyGene + LemnaTec)

A powerful phenotyping equipment known as the PhenoFab is situated there. This platform uses image technologies and moving plant-filled pots or trays through a greenhouse compartment to identify the phenotype and gather digital data. While growing in distinct containers or tray wells, the plants are photographed using VIS (visible light), NIR, and fluorescence imaging equipment at specified time intervals. The facility has climate-controlled rooms that enable experiments under varied growth or stress situations. Fluorescence, visible light, and NIR (near-infrared spectrum) imaging are the foundations of digital trait analysis. RGB images are evaluated using pixel values to identify shape, colour, and other morphological digital properties. The NIR-infrared pictures are used specifically to highlight plant internal structures, water content, or other compositions (like chemical ones). Analysis of the GFP-protein and chlorophyll is made possible by fluorescence imaging. Low variability objects in any spectrum can be resolved using a combination of all imaging methods.

8. The Australian Plant Phenomics Facility

At the Australian Plant Phenomics Facility, digital imaging technology, high-capacity computation, and robotics are coupled to enable automated, high-throughput, non-destructive assessment of plant growth and function ('phenomics'). These resources are housed in a specially constructed building that also has cutting-edge greenhouses, growth chambers, labs, and seed storage facilities.

Fig 7: Photograph of The Australian Plant Phenomics Facility

9. LemnaTec

LemnaTec's phenotyping systems are stationary portal cranes that have the ability to move a measuring support platform over a field area of several hundred square metres at a height of 3 to 6 metres. Visible light, near-infrared, and infrared cameras are used to capture pictures of the field from the top when the platform is moved to various places with great accuracy and reproducibility. The camera data may gain a third dimension thanks to 3D scanners. The system may be utilised even in tropical and subtropical climates because all components are housed and have cooling options. This approach does not have any technological or measurement limitations for studying rival systems with a similar level of depth and attention to detail.

Fig 8: the LemnaTec platform used in [18] equipped with conveyors to move the plants in acquisition booths

10. HTPheno: Image Analysis Pipelines

High-end computational capabilities are required for picture analysis in high-throughput phenotyping research, which is sometimes time-consuming. A single high-throughput experiment generates hundreds of photos. For many biological applications, there are a number of programmes that provide picture editing, image processing, and image analysis [42]. By providing a versatile and extensible picture plug-in that can be utilised for automated picture analysis in high-throughput plant phenotyping, the open-source HTPheno image analysis pipeline generates novel biological discoveries [18]. According to Hartmann *et al*. [18], HTPheno is one such plug-in that offers a flexible image analysis workflow for high-throughput phenotyping.High-end computational capabilities are required for picture analysis in high-throughput phenotyping research, which is sometimes time-consuming. A single high-throughput experiment generates hundreds of photos. For many biological applications, there are several programmes that provide picture editing, image processing, and image analysis [42]. By providing a versatile and extensible picture plug-in that can be utilised for automated picture analysis in high-throughput plant phenotyping, the open-source HTPheno image analysis pipeline generates novel biological discoveries [18]. According to Hartmann et al. [18] HTPheno is one such plug-in that offers a flexible image analysis workflow for high-throughput phenotyping.

11.International Plant Phenomics Network

The International Plant Phenomics Network links organisations that do plant phenomics research worldwide with one another. The phenotyping facilities in Australia, France, Germany, and Canada that are currently its partners include the Australian Plant Phenomics Facility, Ecotron, Centre National de la RechercheScientifique (CNRS), Ecophysiology Laboratory of Plants Under Environmental Stress (LEPSE), INRA, Ju lich Plant Phenotyping Centre (JPPC), and Biotron Experimental Climate Change Research Facility (Biotron).It aims to create, incorporate, and provide novel technologies to analyse plant phenotypes, as well as quality assurance measures in the technologies used for plant phenotyping. It also aims to identify gene functions and their connections to environmental cues, analyse how

environments affect plant structure and function, and evaluate how well plants perform in various environments both in the lab and in the field. This network also encourages the creation of fresh ideas about how plants interact with their surroundings, the adaptation of cutting-edge technology for use in the production of plants, and the performance evaluation of natural plant and ecosystem systems. The International Plant Phenomics Network's main goals are plant breeding for the changing environment, plant performance forecasting in the context of global change, innovative plant production for current and future crops based on an understanding of the complex interactions between plants and their environment and its dynamics, plant performance monitoring in natural systems, and providing a science-based concept and technology to address major challenges of plant performance.

Conclusion

Agricultural development must entail both unprecedented productivity growth and enhanced resource-use efficiency if agricultural yields are to treble by 2050 to achieve production objectives. This is becoming more and more important given the current situation of depleting land and water resources, increasing challenges brought on by climate change, and decreased factor production. Precision phenotyping is not presently capable of keeping up with the advancements in plant genotyping. Due to this, it is challenging to breed cultivars that are trait-specific and to pinpoint the most crucial root and shoot characteristics, which may indicate how a plant will react to input-use efficiency, stress, and shifting environmental factors. Exact phenotyping of pertinent traits is required for modern crop breeding. We have been able to overcome some of these challenges thanks to the development of several HTPPs over the past ten years, but real-time data integration and analysis continue to be a significant barrier. Cost efficiency is still a challenge associated with the creation and application of HTPPs. Therefore, there is an urgent need for the creation of precision phenotyping devices that are less expensive but more efficient and precise. Precision phenotyping requires an interdisciplinary approach rather than merely studying plant physiology or genetics. Regular field phenotyping programmes must incorporate underutilised phenotyping techniques. Similar to this, new fully automated methods that can result in the automatic reconstruction of 3D images will be crucial for exploring resource usage efficiency, as will the phenotyping of root characteristics and their architecture. The ability to improve both current and newly suggested phenotyping platforms will be one of the most crucial factors determining the success of our next crop breeding programmes, along with closing the genome-phenome gap, given the quickly shifting global agricultural research scene.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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