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# Unleashing the potentials of high-throughput phenotyping for accelerating crop breeding

# Alisha, Jogender, Anita Kumari and Renu Munjal

#### Abstract

Biotic and abiotic stress are the major constrains resulting in crop yield reduction and economic losses. It is estimated that the human population will reach to 9 billion by 2050, and current food production must be doubled to meet the needs of the growing population. Therefore, it is the need of hour to increase crop productivity. Advancement in high-throughput phenotyping technologies has progressed significantly in the last decade. These technologies offer precise measurements of desired traits and strategies to screen large population of plants for a particular phenotype under diversified environments employing advanced robotics, high-tech sensors, imaging systems and computing power to unravel the genetic basis of complex traits associated with plant growth and development. Advanced bioinformatics tools further facilitate the analysis of large-scale multi-dimensional, high-resolution data collected through phenotyping from the gene to the whole-plant level under a specific environment and management practices. With the help of integrated approach of genotyping and phenotyping, gene functions and environmental responses can be understood as well. Moreover, it will also help in finding more relevant solutions for the major problem that tend to limit crop production. This review focuses on the recent advances in plant phenomics, various imaging techniques, highlights different field and confined high-throughput technologies for utilization in forward and reverse genetics.

Keywords: Abiotic stress, genotyping, high throughput, imaging, phenotyping

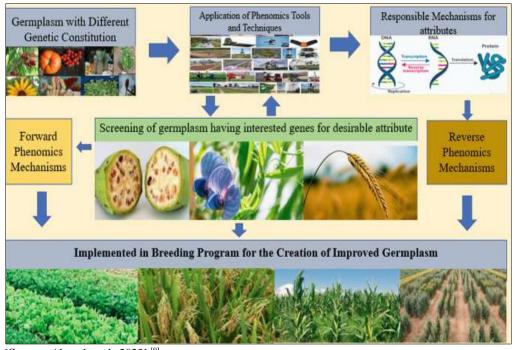
## Introduction

In the era of global climate change, plants are subjected to various stresses, both biotic and abiotic, during their life period. These stresses, individually or in combination, result in significant losses in terms of growth, development and yield and ultimately threaten the survival of the plant. Plants continuously confront harsh environmental conditions in form of abiotic stresses like high/low temperatures, drought, salts, heavy metals, light, flooding and physical wounding resulting in crop yield reduction and economic losses. To ensure that crop production is sufficient to satisfy the needs of a human population that is expected to grow to more than 9 billion by 2050 is a tremendous challenge for plant science and crop improvement (Anonymous, 2017)<sup>[1]</sup>. This goal is challenging primarily because the average rate of crop production increase is only 1.3% per year but it cannot keep pace with population growth. So, we must double the current food production to meet the needs of the growing population (Joshi et al., 2016)<sup>[2]</sup>. In recent times, deteriorating climatic conditions have increased the challenge posed by several biotic and abiotic stresses related to worldwide food production (Pereira, 2016) <sup>[3]</sup>. Climate change result in higher CO<sub>2</sub> concentrations while temperature, heat stress and intermittent rain eventually result in flooding, drought and salt stress (Rosenzweig et al., 2014)<sup>[4]</sup>. Therefore, to minimize the impact of climate change on crop production with a view to feed world's ever-growing population, it is necessary to develop new crop varieties with improved resistance against various biotic and abiotic stresses. Development of crop varieties that can cope up with heat, drought, flood, salinity and other extremes is one of the most important strategies in improving agricultural crop production under changing climatic scenario. Phenotyping is one of the best techniques used for increasing the crop productivity on the basis of better morpho-physiological characters. It has become a major field of research and high throughput phenotyping platforms have been developed to create reproducible environmental scenarios in which the phenotypic responses of multiple genotypes can be analyzed.

#### Phenomics

The recent development in field of plant phenotyping and DNA sequencing techniques, which deal with analysis of large data sets, have given rise to 'phenomics'. The word "phenomics" was coined by Steven A. Garan at the University of Waterloo in 1996. Houle et al. (2010) [5] defined phenomics as the acquisition of multidimensional phenotypic data in an organism ranging from molecule to organ level. Phenomics is the interaction between genotype and environment. It 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 (Close et al., 2011)<sup>[6]</sup>. In 2002, Gerlai used the term "phenomics" to define it as the systematic study of phenotypes on a genome-wide scale. It employs automated non-destructive methods consisting of Robotized delivery of plant to sensors or vice versa and non-invasive sensors for data acquisition.

**Approaches of Phenomics: Forward & Reverse Phenomics** High-throughput phenotyping is one of the most important novel techniques which are used for the development of novel and superior plants, functional analysis of specific genes and for forward & reverse genetic analysis. Moreover, for phenotyping of several distinct lines, including germplasm collection, breeding populations, mapping populations, mutant populations, and under various growth circumstances, high-throughput phenotyping is also required. Plant phenotype is a complex network of interaction between genotype and its prevailing environmental conditions. Plant phenotype and plant functioning may be explained by means of structural traits and physiological traits respectively (Tardieu and Tuberosa, 2010)<sup>[7]</sup>. Understanding the interaction between genotype and phenotype is essential for prediction of plant performance (Soule, 1967; Houle et al., 2010) [8, 5].



[Source: Ahmed et al., 2023] [9]

Fig 1: Illustration of Forward and Reverse Phenomics

There are two different approaches used in high-throughput phenotyping of plants i.e., forward and reverse phenomics (Figure 1). Forward phenomics approach deals with applications of various phenotyping tools and techniques for identification of most promising genotypes from collection of diverse germplasm on basis of visible and valuable traits. It ultimately allows selection of the 'best of the best' elite line/ genotype. It speeds up phenotyping of large number of plants or germplasm lines using automated imaging technology as well as permits rapid identification of desirable traits at prestage and therefore makes it less necessary to grow plants up to the maturity stage in field (Kumar et al., 2015) [10]. It facilitates the screening of thousands of plants (grown in pots) prelabeled with barcodes that run on a conveyor belt and pass through a chamber comprising automated imaging systems for screening interesting traits (Tsaftaris and Noutsos, 2009: Furbank and Tester, 2011) <sup>[11, 12]</sup>. The identified plants with the desirable target traits can then be grown to produce seed for further analysis and breeding. Number of research studies had done in many crops like rice, wheat, sorghum, barley,

Brassica, and Arabidopsis for trait-specific phenotypes (Yang *et al.*, 2020)<sup>[13]</sup>.

On the other hand, reverse phenomics approach deals with understanding of in-depth mechanism that control the traits shown to be valuable (Furbank and Tester, 2011)<sup>[12]</sup>. Further, the identified mechanisms are utilized to exploit new approaches (Kumar et al., 2015)<sup>[10]</sup> and therefore, it helps in developing 'best' genotypes with superior traits/genes. This is achieved by large-scale physiological and biochemical analysis and then linking the data with genes controlling the biochemical or physiological pathway. Once the candidate genes have been identified, the expression pattern of the candidate genes will be compared with other genotypes. Hence, by carefully analyzing the mechanisms underlying particular traits, reverse phenomics enables the exploitation of these mechanisms or the candidate gene(s) associated with the trait, which can then be incorporated into new plant varieties or transferred to other plant species using genetic engineering (Furbank and Tester, 2011)<sup>[12]</sup>.

#### **Plant Phenotyping**

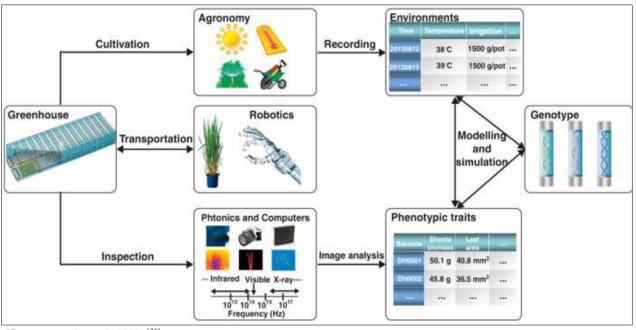
Plant phenotyping is the comprehensive assessment of complex plant traits such as growth, development, tolerance, resistance, architecture, physiology, ecology, yield and the basic measurement of individual quantitative parameters that form the basis for more complex traits (Li *et al.*, 2014). The plant phenotype include these complex traits, and examples of their direct measurement parameters are the root morphology (Walter *et al.*, 2009; Kumar *et al.*, 2014; Flavel *et al.*, 2012; Clark *et al.*, 2011) <sup>[15, 16, 17, 18]</sup>, biomass (Menzel *et al.*, 2009; Golzarian *et al.*, 2011) <sup>[19-20]</sup>, leaf characteristics (Jansen *et al.*, 2009; Arvidsson *et al.*, 2011) <sup>[21-22]</sup>, fruit characteristics (Monforte *et al.*, 2013) <sup>[23]</sup>, yield-related traits (Duan *et al.*, 2011b) <sup>[24]</sup>, photosynthetic efficiency (Bauriegel *et al.*, 2011) and biotic & abiotic stress response (Rao *et al.*, 2013; Balachandran *et al.*, 1997) <sup>[26-27]</sup>.

There are two methods of plant phenotyping: Conventional phenotyping & Modern phenotyping. In conventional phenotyping technique, destructive sampling, manual visual/ instrument aided measurements were used. This technique is very time consuming and labor intensive. While in the modern phenotyping, non-destructive sampling, automatic machines were used. There is a visualization of multi parameter data at one time. One example of modern phenotyping given by Rahman *et al.* (2015) <sup>[28]</sup>. Plants grown

in greenhouses are then conveyed by robotics via conveyer belt to the inspection unit for inspection. There are many kinds of imaging platforms, including visual, thermal, florescence, and others. Data will then be evaluated and interpreted after image processing and when plants are in the field, information is collected by using stationary or mobile sensors such as aerostats, phenocopters, etc., finally data were analyzed and interpretant it.

#### **Phenotype-genotype model**

The main techniques (agronomy, robotics, photonics and computer analyses) needed in plant phenotyping platforms. Plants grown in greenhouses are then transported by robotics via conveyer belt to the inspection chamber for inspection. The inspection chamber, which is the core of the phenotyping platform, carries out the noninvasive, high-throughput screening of plant phenotypic traits using photonics and computers. After image analysis, the quantified traits, environmental data (e.g., illumination, temperature, irrigation, fertilizer) and genotypes are all managed in a database, which produces a 'phenotype–genotype model' (Figure 2) and allows the simulation or predication of responses for special genotypes in different environmental scenarios. (Yang *et al.*, 2013) <sup>[29]</sup>.



[Source: Pasala *et al.*, 2020] <sup>[30]</sup>

**Fig 2:** Phenotype-genotype model

# Advance Technological tools for Plant Phenomics

Plant phenomics is a multidisciplinary science which includes biologists, chemists, physicists, computer scientists, engineers, mathematicians, physiologists, microscopists, geneticists, and plant breeders working together in order to develop novel phenomics tools and methods. Screening of large populations is done in order to exploit genetic variations present in the population for a particular trait like yield potential, drought, salinity, or high-temperature stress tolerance. Parameters of the growth conditions are well defined and precisely monitored. Phenotypic data and metadata descriptions of the experimental conditions are captured for further detailed analysis. These analyses will enable to identify relationships between genotype and phenotype as well as reveal the correlations between seemingly unrelated phenotypes (Schauer *et al.*, 2006; Lu *et al.*, 2008) <sup>[31-32]</sup> and genetic loci (Gerke *et al.*, 2009) <sup>[33]</sup> (Figure 3). The various crucial elements that must be taken into consideration while setting up the high-throughput phenomics platform are high-resolution imaging devices, automated transport for moving plants for imaging, sophisticated imaging algorithms, and data from another relevant research.

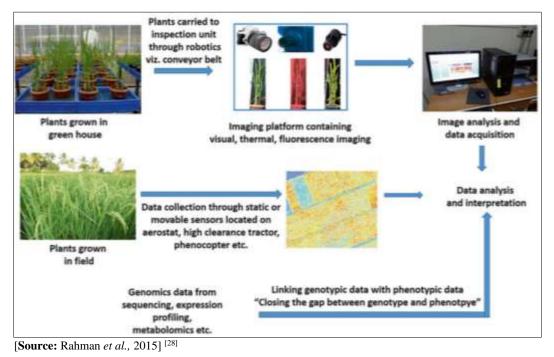


Fig 3: Flowchart of process of high-throughput phenotyping

## **High-throughput imaging techniques**

High-throughput plant phenomics includes different imaging techniques that allow phenotyping of large plant populations at each plant level within a short interval of time. Imaging plant is not just taking or clicking picture. Its main aim to measure a phenotype quantitatively through interaction between light and plants such as reflected photons, absorbed photons or transmitted photons. It is used to quantify complex traits in controlled environmental systems or in the field. These all-imaging techniques are artificial sensors but our human eye is a natural sensor. We see an object, when light reflecting from it falls on our eye retina and our brain recognizes the object. The sensitivity of our eye is limited to visible region of electro-magnetic spectrum. Some phenomics imaging techniques are 3D imaging, infrared imaging, thermographic imaging, fluorescence imaging, hyperspectral imaging, visible light scanning, spectroscopy, chlorophyll fluorescence, X ray/ CT scan and magnetic resonance (Sozzani et al., 2014)<sup>[34]</sup>.

Table 1: List of different imaging techniques

Technique	Sensor	Parameters phenotyped	
<sup>a</sup> Visible light imaging	Standard visible light camera	Plant biomass; root architecture; growth dynamic; seed germination rate; vegetation indices; plant height; plant structure; morphological traits; panicle traits etc.	
<sup>a</sup> Thermal imaging	Near-infrared camera	Leaf area index; severity of disease; insect infestation of seed; seed composition; canopy, shoot and leaf temperature; transpiration and stomatal conductance; plant water status etc.	
<sup>a</sup> Fluorescence imaging	Fluorescence camera	Photosynthesis activity; quantum yield; severity of leaf disease; health status of leaf; non- photochemical quenching; chlorophyll conductance; pigment composition, etc.	
<sup>a</sup> Hyperspectral imaging	Hyperspectral camera, thermal camera, spectrometer Leaf and canopy water content; leaf growth; leaf and panicle health status; quality gra pigment composition; vegetation and water indices; soil cover status; photosynthesis ra levels of phytochemicals, etc.		
<sup>a</sup> 3D imaging	Stereo cameras, time-of-flight cameras	Canopy and shoot structure; root architecture; plant height, etc.	
<sup>a</sup> Laser imaging	Wide range laser scanning instruments	Shoot, root and canopy structure; shoot biomass; plant height; leaf angle distribution, etc.	
<sup>b</sup> Magnetic resonance imaging	Magnetic resonance imager	Water content; morphometric parameters; plant health status; metabolic study, etc.	

a: Applicable in controlled and field conditions; b: Applicable in controlled conditions

[Source: Jangra *et al.*, 2021] <sup>[35]</sup>

## Visible light imaging

Visible light imaging technology is widely used in plant science. Image captured in the visible spectrum are widely used for monitoring plant growth and development. Within wavelength (400-700 nm) perception identical to human eye, two-dimensional (2D) images captured by digital cameras can be used to analyze various plant traits such as shoot biomass (Tackenberg, 2007; Golzarian et al. 2011) [36, 20], yield-related traits (Duan et al. 2011a, b) [37], leaf morphology (Bylesjo et al., 2008) <sup>[38]</sup>, panicle traits (Ikeda et al., 2010) <sup>[39]</sup>, and root architecture (Pascuzzi et al., 2010)<sup>[40]</sup>. Humplik et al. (2015) <sup>[41]</sup> reviewed on the automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses and it found that the 18 DAG old soil-grown Arabidospis plants were treated with 250 mM NaCl (salt-stressed) and water (control) by using three different types of imaging system i.e. RGB, hyperspectral and Chl fluorescence imaging and after 48 hours, there is an analysis of arabidopsis by

different sensors for comparison in: morphology (top-view RGB imaging can be used for computation of rosette area or shape parameters), spatial distribution of vegetation index reflecting changes in the chlorophyll content (NDVI) provided by VIS/NIR hyperspectral camera, and the changes in maximal quantum yield of PSII photochemistry for a dark-adapted state ( $\Phi$ Po, also referred as FV/FM) reflecting the photosynthetic activity of the plants.

#### Fluorescence imaging

The absorbance of light by a compound at a particular wavelength and further emission of low wavelength ligh, is termed fluorescence. Fluorescence imaging flashes blue wavelength (<500 nm) light on the plants and they emit fluorescence light at 600-750 nm in the red region of the spectrum. The difference in fluorescence are photographed and converted in to false color signals using computer software to analyze them (Weirman, 2010)<sup>[42]</sup>. Traits measured by fluorescence imaging technique are Photosynthetic efficiency, Leaf health status, Shoot architecture, non-photochemical quenching etc. Hairmansis et al., 2014 [43] worked on the RGB and fluorescent imaging technique to find out tolerant rice variety under salinity. Two rice cultivars were taken i.e. IR64 and Fatmawati. The cultivar Fatmawati appears to be significantly more salt sensitive than IR64, showing considerable shoot senescence (23%) when exposed to 200 mM NaCl for 20 d. IR64, in contrast, exhibited little shoot senescence (4%), even under very high NaCl concentrations. While there was an increase in shoot senescent area that corresponded to increasing salt concentrations, both cultivars had little senescence at moderate salinity levels. So, it concludes that IR64 is a salt tolerant variety.

# Infrared Thermal based imaging

Thermal imaging allows for the visualization of infrared radiation, indicating an object as the temperature across the object's surface. The range of thermal cameras are  $3-14\mu m$  and the most commonly used wavelengths for thermal

imaging are 3-5 um or 7-14 um. Infrared imaging devices use two main wavelength ranges, Near infrared (3-5 µm) and far infrared (7-14 µm). The thermal sensitivity of smaller wavelength is 3-5 um, which makes it higher than hat of wave length at 7-14 um because smaller wavelength correspond to higher energy level. Traits measured by infrared thermal based imaging technique are:-canopy or leaf temperature, insect infestation of grain. Canopy temperature measured by Infrared thermometry i.e. represent the temperature of leaf while the infrared thermography represent the temperature and colored image of particular plant or whole. Kwon et al. (2015) <sup>[44]</sup> used infra-red thermography technique for phenotyping of plants for drought and salt tolerance. They successfully distinguished the difference between the tolerant lines and sensitive lines with great correlation with physiological traits like relative water content and stomatal conductance. Deery et al. (2016)<sup>[45]</sup> did a field experiment of canopy temperature using Airborne thermography in wheat.

#### Spectroscopy Imaging

Spectroscopy imaging is the outcome of the interaction of solar radiation with plants through multispectral and hyperspectral cameras. In contrast to visible and infrared imaging, hyperspectral imaging divides images into bands, thus creating a large fraction of the electromagnetic spectrum in the images (Yang *et al.*, 2013) <sup>[29]</sup>. Traits measured by spectroscopy imaging technique are greenness, biomass, canopy chlorophyll content, leaf and canopy senescence, plant water status, photosynthetic status, LAI or NDVI.

## **3-D** Mapping of plants

Digital photos of the top and sides of plants are combined into a 3-D image. Traits measured by 3-D mapping of plants are Shoot mass, leaf no., leaf shape, leaf colour, leaf angle and leaf health. Sensor used in 3-D mapping is LIDAR (laser scanner). It create accurate and detailed 3-D models by light projections and laser range scanners. This technique is expensive, complex and require longer imaging times.

Imaging technique	Crop	Traits studied	References
Visible light imaging	Barley	Shoot biomass under salinity	Golzarian et al. (2011) <sup>[20]</sup>
	Rice	Yield traits like number of total spikelets, number of filled spikelets, grain length, grain width, and 1,000-grain weight	Duan et al. (2011a, b) <sup>[37]</sup>
		Panicle traits like panicle length and number of various branches and grain number	Ikeda et al. (2010) <sup>[39]</sup>
		Root system architecture	Pascuzzi <i>et al.</i> (2010) <sup>[40]</sup> ; Clark <i>et al.</i> (2011) <sup>[18]</sup>
Thermo graphic imaging	Rice	Leaf area index	Sakamoto <i>et al.</i> (2011) <sup>[46]</sup> ; Shibayama <i>et al.</i> (2011a, b) <sup>[47]</sup>
	Maize	Starch, protein and oil content in kernel for GWAS	Cook et al. (2012) <sup>[49]</sup>
	Barley, wheat	Shoot temperature under water deficit condition	Munns et al. (2010) <sup>[50]</sup>
		Osmotic components of salinity tolerance	Sirault <i>et al.</i> (2009) <sup>[51]</sup>
Hyperspectral imaging	Rice	Leaves and canopy damaged caused by biotic and abiotic stresses	Huang <i>et al.</i> (2012) <sup>[52]</sup> ; Munns <i>et al.</i> (2010) <sup>[50]</sup>
		Leaf growth and nitrogen status	Nguyen and Lee (2006) <sup>[53]</sup>
		Panicle health status under biotic stresses	Liu et al. (2010) <sup>[54]</sup>
	Wheat	Kernel damage by various insects	Singh et al. (2010) [55]
Chlorophyll fluorescence	Wheat	Early detection of disease incidence	Moshou <i>et al.</i> (2005) <sup>[56]</sup>
	Barley, wheat	Shoot temperature under water deficit	Munns et al. (2010) <sup>[50]</sup>
V	Wheat	Root growth parameters under phosphorous fertilization	Flavel <i>et al.</i> (2012) <sup>[17]</sup>
X-ray	Wheat, rapeseed	Root growth and architecture under root/soil interactions	Gregory et al. (2003) [57]

**Table 2:** Applications of imaging techniques in field crops

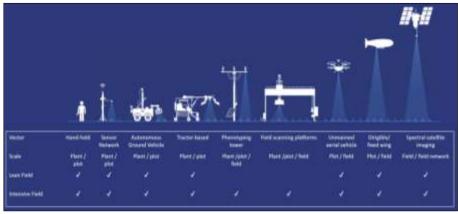
[Source: Rahman et al., 2015] [28]

#### **Field-Based Plant Phenomics**

Significant advancements have been made in field of automated phenotyping platforms in form of application of robotics, new sensors, and advanced imaging technologies in growth chambers and glasshouses (Granier et al., 2006; Jansen et al., 2009; Furbank and Tester, 2011) [58, 21, 12]. Providing quantitative information on the dynamic responses of plants to their natural environment is the main objective of plant phenomics. Screening the plants in greenhouse/controlled environment suffer from a number of limitations like changes in solar radiation, wind speed and evaporation rates, limited soil volume, nutrient availability in pots, and interaction with mutualistic, parasitic or competitor organisms, etc. In addition, when plants are screened in controlled environments for a specific trait, the interaction between changing environment and plant phenotype is greatly missed and fail to characterize the responses relevant to the

## field conditions (Poorter et al., 2012)<sup>[59]</sup>.

Therefore, screening and identification of germplasm for a desirable trait is done best under such field conditions of soil, climate, and biotic stress agents where final varieties will be grown. However, researchers are continuously putting their efforts to develop such reliable techniques for field-based phenotyping (FBP) which screen at a larger scale and predict an accurate description of trait expression in cropping system. Furthermore, field measurements serve as a significant test for the relevance of the laboratory and greenhouse approaches. For FBP, airplane and satellite-based systems are used at field scale, but studies using proximal (close-range) sensing are typically the only method that can provide reliable data with sufficient resolution, multiple angles, and illumination control, as well as at a closer proximity to the target to the sensors (White *et al.*, 2012) <sup>[60]</sup>.



[Source: Morisse *et al.*, 2022] <sup>[61]</sup>

Fig 4: Overview of different field phenotyping platforms

#### Relevance of high-throughput phenotyping technology Screening and identification of Abiotic Stress

Abiotic stresses such as drought, salinity, submergence and high & low temperature, reduces crop growth and biomass leading to yield loss in agricultural crops worldwide. The response of plants against these stresses is sophisticated and dynamic and it involves a complex crosstalk between multiple pathways at different regulatory levels (Saito and Matsuda 2010) <sup>[62]</sup>, phenotyping for tolerance against these abiotic stresses is often a big challenge. Screening of large mapping population or a collection of germplasms for QTL analysis or association mapping of abiotic stress tolerance is a time consuming and impractical task. But with the advancement of high-throughput phenotyping techniques, it has now become possible to screen multiple traits for huge population size nondestructively under stress conditions. Abiotic stresses such as drought and salinity etc. are highly associated with phenotypic, biochemical and physiological changes, which are outlined in Table 3.

Crop	Stress	Automated techniques	
Arabidopsis	Heat, Drought, chilling	RGB (top view) TLCFIM	
Barley	Drought, salt	RGB (multiple view), hyperspectral NIR, SLCFIM	
Grapevines	Drought	RGB (multiple view)	
Tomato	Drought	RGB (multiple view), hyperspectral NIR, SLCFIM Lemna Tech	
Rice	Salt	RGB (multiple view) SLCFIM	
Sorghum	Nutrient deficiency	RGB (multiple view), hyperspectral NIR	
Bean	Nutrient deficiency	RGB (top view), thermoimaging, TLCFIM	
Pea	Cold	RGB (multiple view) KCFIM	

[Source: Jangra et al., 2021] <sup>[35]</sup>

#### Study of various physiological processes in plants

The photosynthesis plants have two major photosynthetic mechanisms, *i.e.*, C3 and C4. Researchers want to replace the C3 pathway of crop species with a more efficient C4 mechanism because C4 plants can concentrate carbon dioxide inside the leaf and photosynthesize more efficiently compared

to C3 plants (Von Caemmerer *et al.*, 2012) <sup>[63]</sup>. But the major limiting factor in photosynthetic performance is the inefficiency of the enzyme Rubisco. Some plants have better Rubisco efficiency than others. Using phenomics, researchers are searching through thousands of wheat varieties with a better performing Rubisco and higher rates of photosynthesis

that can grow well under nutrient deficiency, drought and salinity (Tackenberg 2007; Baker 2008) <sup>[36, 64]</sup>.

#### Rapid and efficient screening for elite mutants

Remote sensing technology enables plant researchers to analyze a large number of plants in field conditions. Measurements can be taken on large population of plants at once and over a whole growing season. Some examples of phenomics field technology are phenonet sensor network, phenomobile, phenotower and multicopter. These technologies are also used in detection and monitoring of disease epidemics in the field and root attack by pathogens, facilitating the screening of germplasm and modeling of biomass production (Miyao *et al.*, 2007)<sup>[65]</sup>.

#### **High-Throughput Plant Phenotyping Facilities in India**

Phenotyping facilities are becoming important in developing countries like India. Recently several phenotypic platforms have been established in our country. Plant Phenomics National Facility at ICAR-IIHR (Indian Institute of Horticulture Research) was inaugurated on November 2015 and this advanced facility supports the research activities engaged in non-destructive identification of tolerant genotypes under adaptation strategies to manage climate change impacts on horticultural crops. Phenospex highthroughput field phenotyping facility (Field Scan) was inaugurated at ICRISAT, India, in April 2014. This platform has a capacity of 10,000 of plants with a throughput of 5000 plants/h. Available at ICRISAT, Leasy Scan is a phenotyping platform that uses Plant Eye camera and captures 3D images. This equipment is capable of scanning 3200-4800 plot/2 h. High-throughput phenotyping facilities were inaugurated at Central Research Institute for Dryland Agriculture (CRIDA) in July, 2014. This facility was installed under the National Initiative on Climate Resilient Agriculture (NICRA) project launched by ICAR. This phenotyping-platform allows quantitative and non-destructive analysis of crop varieties and germplasms under controlled environmental conditions. ICAR through National Agricultural Science Fund (NASF) established a state-of-the art plant phenomics facility at the Indian Agricultural Research Institute, New Delhi (Figure 5). This facility is the largest in India and one of best facility in terms of analytical capabilities among the public funded Institutions in the world. The centre also comprises of "Climate Controlled Facility" with 8 different greenhouse chambers with precision control of temperature and CO<sub>2</sub>. This will be highly useful to study the interactive effects of elevated CO<sub>2</sub> with heat and other climatic stress factors. The facility has four hi-tech climate-controlled greenhouses for cultivation of plants in defined environmental conditions. For plant cultivation, the facility is equipped with 1200 plant carriers with RFID chip tag. The plant carrier on moving field conveyer system randomizes plants within the greenhouse and carries plants for automated weighing and watering, and imaging at various imaging platforms. The facility has 5 automated weighing and watering stations for precise imposition of drought stress to plants and to measure transpirational water loss and water use efficiency of plants (PlantPhenomicsCentre\_inauguration\_News\_13102017.pdf).



[Source: PlantPhenomicsCentre\_inauguration\_News\_13102017.pdf]

Fig 5: A birds-eye view of Nanaji Deshmukh Plant Phenomics Centre at ICAR-IARI, New Delhi

# **Conclusion and future prospectives**

Phenotyping is an efficient tool to screen and select tolerant germplasm under different abiotic stresses in a very less time as compared to conventional phenotyping. The use of noninvasive high throughput sensors have really enhanced the capability of phenomics platform to acquire thousands of phenotypic features, as compared to few traits measured by conventional methods. Use of phenomics for analytical breeding is expected to break the barriers of yield and adaptability of crops to stress environment. Majority of the available plant phenomics technologies are restricted to controlled environments, which poses a challenge for collecting phenotypic data in a dynamic microclimate. For producing accurate, precise, multi-dimensional and reliable phenotypic data, agricultural germplasm, breeding lines, and mutant populations in field banks may be the next target for field phenomics imaging technologies. Recently, various

powerful field imaging technology sensors, such as phenonet sensor network, phenomobile, phenotower and multicopter, were used to produce realistic data. For greater usage of these plant phenomics technologies to identify the climate-resilient varieties, user-friendly, cost-effective equipment coupled with basic image processing software and simple statistical analysis programmes is required.

#### Conflict of interest: None

#### References

- 1. Anonymous. United Nations Department of Economic and Social Affairs Population Division; c2017. Available online: http://www.unpopulation.org.
- 2. Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, *et al.* Transcription factors and plants response to drought stress: current understanding and future

directions. Frontiers in Plant Science. 2016;7:1029.

- 3. Pereira A. Plant abiotic stress challenges from the changing environment. Frontiers in Plant Science. 2016;7:1123.
- 4. Rosenzweig C, Elliott J, Deryng D, Ruane AC, Müller C, Arneth A, *et al.* Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proceedings of the National Academy of Sciences. 2014;111(9):3268-3273.
- Houle D, Govindaraju DR, Omholt S. Phenomics: the next challenge. Nature Reviews Genetics. 2010;11:855-866.
- 6. Close T, Riverside UC, Last R. National Science Foundation Phenomics: genotype to phenotype, a report of the NIFA-NSF phenomics workshop; c2011.
- 7. Tardieu F, Tuberosa R. Dissection and modeling of abiotic stress tolerance in plants. Current Opinion in Plant Biology. 2010;13:206-212.
- 8. Soule M. Phenetics of natural populations I. Phenetic relationships of insular populations of the side-blotched lizard. Evolution. 1967;21:584-591.
- Ahmed HGMD, Zeng Y, Fiaz S, Rashid AR. Applications of High-Throughput Phenotypic Phenomics. In: Prakash, C.S., Fiaz, S., Nadeem, M.A., Baloch, F.S., Qayyum, A. (eds) Sustainable Agriculture in the Era of the OMICs Revolution. Springer, Cham; c2023. https://doi.org/10.1007/978-3-031-15568-0\_6.
- 10. Kumar J, Pratap A, Kumar S. Plant Phenomics: An Overview. Phenomics in crop plants: Trends, Options and Limitations (eds.); c2015.
- 11. Tsaftaris S, Noutsos C. Plant phenotyping with low-cost digital cameras and image analytics; in Information Technologies in Environmental Engineering (Springer: Berlin/Heidelberg, Germany); c2009. p. 238-251.
- Furbank RT, Tester M. Phenomics-technologies to relieve the phenotyping bottleneck. Trends Plant Science. 2011;16:635-644.
- 13. Yang W, Feng H, Zhang X, Zhang J, Doonan JH, Batchelor WD, *et al.* Crop phenomics and High-Throughput phenotyping: Past decades, current challenges, and future perspectives. Molecular Plant. 2020;13:187-214.
- Li L, Zhang Q, Huang D. A review of imaging techniques for plant phenotyping. Sensors. 2014;14(11):20078-20111.
- 15. Walter A, Silk WK, Schurr U. Environmental effects on spatial and temporal patterns of leaf and root growth. Annual Review of Plant Biology. 2009;60:279-304.
- 16. Kumar P, Huang C, Cai J, Miklavcic SJ. Root phenotyping by root tip detection and classification through statistical learning. Plant and Soil. 2014;380(1-2):193-209.
- 17. Flavel RJ, Guppy CN, Tighe M, Watt M, McNeill A, Young IM. Non-destructive quantification of cereal roots in soil using high-resolution X-ray tomography. Journal of Experimental Botany. 2012;63(7):2503-2511.
- 18. Clark RT, MacCurdy RB, Jung JK, Shaff JE, McCouch SR, Aneshansley DJ, *et al.* Three-dimensional root phenotyping with a novel imaging and software platform. Plant Physiology. 2011;156(2):455-465.
- 19. Menzel MI, Tittmann S, Buehler J, Preis S, Wolters N, Jahnke S, *et al.* Non-invasive determination of plant biomass with microwave resonators. Plant, Cell &

Environment. 2009;32(4):368-379.

- 20. Golzarian MR, Frick RA, Rajendran K, Berger B, Roy S, Tester M, *et al.* Accurate inference of shoot biomass from high-throughput images of cereal plants. Plant Methods. 2011;7:2.
- 21. Jansen M, Gilmer F, Biskup B, Nagel KA, Rascher U, Fischbach A, *et al.* Simultaneous phenotyping of leaf growth and chlorophyll fluorescence via grow screen fluoro allows detection of stress tolerance in Arabidopsis thaliana and other rosette plants. Functional Plant Biology. 2009;36:902-914.
- 22. Arvidsson S, Pérez-Rodríguez P, Mueller-Roeber B. A growth phenotyping pipeline for Arabidopsis thaliana integrating image analysis and rosette area modeling for robust quantification of genotype effects. New Phytologist. 2011;191(3):895-907.
- 23. Monforte AJ, Diaz A, Caño-Delgado A, Van Der Knaap E. The genetic basis of fruit morphology in horticultural crops: lessons from tomato and melon. Journal of Experimental Botany. 2013;65(16):4625-4637.
- 24. Duan L, Yang W, Huang C, Liu Q. A novel machinevision-based facility for the automatic evaluation of yield-related traits in rice. Plant Methods. 2011b;7(1):44.
- 25. Bauriegel E, Giebel A, Herppich WB. Hyperspectral and chlorophyll fluorescence imaging to analyse the impact of Fusarium culmorum on the photosynthetic integrity of infected wheat ears. Sensors. 2011;11(4):3765-3779.
- 26. Rao NKS, Laxman RH. Phenotyping horticultural crops for abiotic stress tolerance. In Climate-Resilient Horticulture: Adaptation and Mitigation Strategies; c2013. p. 147-157.
- 27. Balachandran S, Hurry VM, Kelley SE, Osmond CB, Robinson SA, Rohozinski J, *et al.* Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. Physiologia Plantarum. 1997;100(2):203-213.
- Rahman H, Ramanathan V, Jagadeeshselvam N, Ramasamy S, Rajendran S, Ramachandran M, *et al.* Phenomics: technologies and applications in plant and agriculture. Plant Omics: The Omics of Plant Science; c2015. p. 385-411.
- 29. Yang W, Duan L, Chen G, Xiong L, Liu Q. Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies. Current Opinion in Plant Biology. 2013;16(2):180-187.
- Pasala R, Pandey BB. Plant phenomics: High-throughput technology for accelerating genomics. Journal of Biosciences. 2020;45(1):111.
- Schauer N, Semel Y, Roessner U, Schauer N, Semel Y, Roessner U, *et al.* Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nature Biotechnology. 2006;24:447-454.
- 32. Lu Y, Savage LJ, Ajjawi I, Imre KM, Yoder DW, Benning C, *et al.* New connections across pathways and cellular processes: industrialized mutant screening reveals novel associations between diverse phenotypes in Arabidopsis. Plant Physiology. 2008;146:1482-1500.
- Gerke J, Lorenz K, Cohen B. Genetic interactions between transcription factors cause natural variation in yeast. Science. 2009;323:498-501.
- 34. Sozzani R, Busch W, Spalding EP, Benfey PN. Advanced

imaging techniques for the study of plant growth and development. Trends Plant Science. 2014;19:304-310.

- 35. Jangra S, Chaudhary V, Yadav RC, Yadav NR. Highthroughput phenotyping: a platform to accelerate crop improvement. Phenomics. 2021;1(2):31-53.
- 36. Tackenberg O. A new method for non-destructive measurement of biomass, growth rates, vertical biomass distribution and dry matter content based on digital image analysis. Annals of botany. 2007;99(4):777-783.
- 37. Duan L, Yang W, Bi K, Chen S, Luo Q, Liu Q. Fast discrimination and counting of filled/unfilled rice spikelets based on bi-modal imaging. Computers and Electronics in Agriculture. 2011a;75(1):196-203.
- Bylesjo M, Segura V, Soolanayakanahally RY, Rae AM, Trygg J, Gustafsson P, *et al.* LAMINA: a tool for rapid quantification of leaf size and shape parameters. BMC Plant Biology. 2008;8:82.
- 39. Ikeda M, Hirose Y, Takashi T, Shibata Y, Yamamura T, Komura T, *et al.* Analysis of rice panicle traits and detection of QTLs using an image analyzing method. Breeding Science. 2010;60(1):55-64.
- 40. Pascuzzi Iyer AS, Symonova O, Mileyko Y, Hao Y, Belcher H, Harer J, *et al.* Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. Plant Physiology. 2010;152:1148-1157.
- Humplík JF, Lazár D, Husičková A, Spíchal L. Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses-a review. Plant Methods. 2015;11:29.
- 42. Weirman A. Plant phenomics teacher resource; c2010. http://www.plantphenomics.org.au/files/teacher/Final\_Ph enomics\_for\_word\_with\_imagsdoc.
- 43. Hairmansis A, Berger B, Tester M, Roy SJ. Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. Rice. 2014;7(1):16.
- 44. Kwon TR, Kim KH, Yoon HJ, Lee SK, Kim BK, Siddiqui ZS. Phenotyping of plants for drought and salt tolerance using infra-red thermography; c2015.
- 45. Deery DM, Rebetzke GJ, Jimenez-Berni JA, James RA, Condon AG, Bovill WD, *et al.* Methodology for highthroughput field phenotyping of canopy temperature using airborne thermography. Frontiers in Plant Science. 2016;7:1808.
- 46. Sakamoto T, Shibayama M, Kimura A, Takada E. Assessment of digital camera-derived vegetation indices in quantitative monitoring of seasonal rice growth. ISPRS Journal of Photogrammetry and Remote Sensing. 2011;66:872-882.
- 47. Shibayama M, Sakamoto T, Takada E, Inoue A, Morita K, Takahashi W, *et al.* Estimating paddy rice leaf area index with fixed point continuous observation of near infrared reflectance using a calibrated digital camera. Plant Production Science. 2011a;14:30-46.
- 48. Shibayama M, Sakamoto T, Takada E, Inoue A, Morita K, Yamaguchi T, *et al.* Regression-based models to predict rice leaf area index using biennial fixed point continuous observations of near infrared digital images. Plant Production Science. 2011b;14:365-376.
- 49. Cook JP, McMullen MD, Holland JB, Tian F, Bradbury P, Ibarra JR, *et al.* Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels. Plant Physiology. 2012;158:824-834.
- 50. Munns R, James RA, Sirault XR, Furbank RT, Jones HG. New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. Journal of

https://www.thepharmajournal.com

Experimental Botany. 2010;61:3499-3507.

- 51. Sirault XRR, James RA, Furbank RT. A new screening method for osmotic component of salinity tolerance in cereals using infrared thermography. Functional Plant Biology. 2009;36:970-977.
- 52. Huang JR, Liao HJ, Zhu YB, Sun JY, Sun QH, Liu XD. Hyperspectral detection of rice damaged by rice leaf folder (*Cnaphalocrocis medinalis*). Computers and Electronics in Agriculture. 2012;82:100-107.
- 53. Nguyen HT, Lee BW. Assessment of rice leaf growth and nitrogen status by hyperspectral canopy reflectance and partial least square regression. European Journal of Agronomy. 2006;24:349-356.
- 54. Liu ZY, Shi JJ, Zhang LW, Huang JF. Discrimination of rice panicles by hyperspectral reflectance data based on principal component analysis and support vector classification. Journal of Zheijang University Science B-Biomedicine & Biotechnology. 2010;11:71-78.
- 55. Singh CB, Jayas DS, Paliwal J, White NDG. Identification of insect-damaged wheat kernels using short-wave near-infrared hyperspectral and digital colour imaging. Computers and Electronics in Agriculture. 2010;73:118-125.
- 56. Moshou D, Bravo C, Oberti R, West J, Bodria L, McCartney A, *et al.* Plant disease detection based on data fusion of hyper-spectral and multispectral fluorescence imaging using Kohonen maps. Real-Time Image. 2005;11:75-83.
- 57. Gregory PJ, Hutchison DJ, Read DB, Jenneson PM, Gilboy WB, Morton EJ. Non-invasive imaging of roots with high resolution X-ray micro-tomography. Plant Soil. 2003;255:251-259.
- 58. Granier C, Aguirrezabal L, Chenu K, Cookson SJ, Dauzat M, Hamard P, *et al.* Phenopsis, an automated platform for reproducible phenotyping of plant responses to soil water deficit in *Arabidopsis thaliana* permitted the identification of an accession with low sensitivity to soil water deficit. New Phytologist. 2006;169:623-635.
- 59. Poorter H, Fiorani F, Stitt M, Schurr U, Finck A, Gibon Y, *et al.* The art of growing plants for experimental purposes: a practical guide for the plant biologist. Functional Plant Biology. 2012;39:821-838.
- 60. White JW, Andrade-Sanchez P, Gore MA, Bronson KF, Coffelt TA, Conley MM, *et al.* Field-based phenomics for plant genetics research. Field Crops Research. 2012;133:101-112.
- 61. Morisse M, Wells DM, Millet EJ, Lillemo M, Fahrner S, Cellini F, *et al.* A European perspective on opportunities and demands for field-based crop phenotyping. Field Crops Research. 2022;276:108371.
- 62. Saito K, Matsuda F. Metabolomics for functional genomics, systems biology, and biotechnology. Annual Review of Plant Biology. 2010;61:463-489.
- 63. Von Caemmerer S, Quick WP, Furbank RT. The development of C4 rice: Current progress and future challenges. Science. 2012;336:1671-1672.
- 64. Baker NR. Chlorophyll fluorescence: A probe of photosynthesis *in vivo*. Annual Review of Plant Biology. 2008;64:89-113.
- 65. Miyao A, Iwasaki Y, Kitano H, Itoh J, Maekawa M, Murata K, *et al.* A large-scale collection of phenotypic data describing an insertional mutant population to facilitate functional analysis of rice genes. Plant Molecular Biology. 2007;63:625-635.