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Review Paper

Phenomics: The next frontier in climate resilient crop varietal development: a review

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ABSTRACT

Global agriculture is facing major challenges to ensure global food security, such as the need to breed high-yielding crops adapted to future climates. Plant phenotyping provides a set of tools to help researchers better understand gene function and environmental processes. Reliable, autonomous, multipurpose, and high-throughput phenotypic technologies are becoming more significant tools in breeding programs for rapid progression of genetic gain. With the rapid advancement of high-throughput phenotyping technology, research has entered a new era known as phenomics. Here we present an overview of phenomics research, focused on the collecting of phenotypic data utilizing various sensors. Finally, we discuss the crop phenomics' challenges and prospects in order to make recommendations for developing new methods for mining genes associated with important agronomic traits and proposing new solutions for precision agriculture.

Keywords: Forward phenomics, high-throughput phenotyping, phenotype, reverse phenomics

Food and feed shortages, resource scarcity, climate change, and energy use are some of the difficulties that we face as a result of our reliance on plants. Crop production will have to double until 2050 to meet predicted world population production demands (Ray *et al*., 2013). Crop production demand is predicted to expand at a pace of 2.4 percent per year, but crop yields are only expected to improve at a rate of 1.3 percent per year. Furthermore, output yields have remained stable on up to 40% of cereal-growing area (Fischer and Edmeades, 2010). Genetic improvements in crop performance plays the key role in improving crop productivity, but the current rate of development will not be enough to meet the needs of sustainability and food security. Selections based on genotypic information are given more attention in molecular breeding strategies, but phenotypic data are still required (Jannick *et al*., 2010; Araus and Cairns, 2014). In transgenic investigations, phenotyping is required to improve the efficiency of selection and the reproducibility of results (Gaudin *et al*., 2013; Yang *et al*., 2014; Feng *et al*., 2017). Given that molecular breeding populations can include anywhere from 200 to 10,000 lines, but the ability to accurately and quickly define hundreds of lines simultaneously remains a challenge (Lorenz *et al*., 2011; Araus and Cairns, 2014). There appears to be a phenotyping bottleneck, in comparison to the large genetic data, impeding progress in understanding the genetic basis of complex traits. Reliable, automatic, and high-throughput phenotypic technologies were urgently needed to break this bottleneck and improve the efficiency of molecular breeding. Plant breeding requires the assessment of attributes that are significant commercially. The basic method of manual measuring or scoring used in traditional phenotyping is laborious, time-consuming, and tiresome. Speed breeding and precise farming have raised the bar for phenotyping's efficiency, precision, reliability, and innovation. First, the cornerstone is increased precision and reliable phenotyping. Second, non-destructive, fast and reproducible phenotyping, like senescence dynamics, are becoming more and more in demand. Third, it is hard to assess novel, high-dimensional and hidden phenotypes using conventional techniques

(Liu.F *et al*., 2021;Xiao F *et al*., 2021) . These technologies would provide breeding scientists with new insights in selecting new species to adapt to resource shortages and global climate change Plant phenomics, which is defined as the collection of multi-dimensional phenotypic data at different levels from cell to organ to plant to population level, has transformed from an emerging niche to a vibrant study topic during the last decade (Houle *et al*., 2010; Dhondt *et al*., 2013; Lobos *et al*., 2017). Crop phenotypes are the complex interactions between genotypes (G) and a range of environments (E) (Xu, 2016). This interaction has an impact on plant growth and development, as evaluated by structural traits at the cellular, tissue, organ, and plant levels, as well as plant function, as measured by physiological attributes. External phenotypes including as morphology, biomass, and yield performance are determined by these internal phenotypes (Houle *et al*., 2010; Dhondt *et al*., 2013). Phenomics research combines agronomy, life sciences, mathematics and engineering , as well as high-performance computing and artificial intelligence technology, to investigate a wide range of phenotypic information about crop growth in a complex environment, with the ultimate goal of building an effective technical system able to phenotype crops in a high-throughput, multi-dimensional, big-data, intelligent, and automatically measuring manner, and create a tool by integrating huge data obtained from a multimodality, multi-scale, phenotypic + environmental + genotypic conditions and develop novel approaches for mining genes linked with essential agronomic features.

What is phenomics: Michael Soulé, an evolutionary biologist used the term phenome to refer to the phenotype as a whole. Phenomics is now defined as the acquisition of high-dimensional phenotypic data on an organismal wide scale. Although phenomics is described in terms of genomes, the comparison is deceptive in one way. We can almost completely characterize a genome, but not a phenome, because phenomes have far more information than genomes. Phenotypes fluctuate from cell to cell and from moment to moment, making it impossible to fully characterize them. Prioritizing what to measure and striking a balance between exploratory and explanatory goals will always be part of phenomics. We also require a phenomic conceptual framework to analyse high-dimensional phenomic data, especially when it spans numerous levels of organization. Fortunately, this framework may draw on well-established intellectual traditions in quantitative genetics, evolutionary biology, epidemiology, and physiology to analyse phenotypic data. These fields give researchers the ability to account for many sources of variation and separate causes from correlations. In fact, phenomics is employed in the same way as genomics is, however it is not the same as genomics. Comprehensive genome characterization is attainable in genomics, but complete phenome characterization is difficult in phenomics due to changes in phenotypic expression of traits over time and environmental conditions (Houle *et al*. 2010).

Phenotype vs Phenomics : A plant's phenotype is defined by its morphological, biochemical, physiological, and molecular characteristics. To describe these qualities, various parameters are measured. The words 'genotype' and 'phenotype' were coined by Johannsen (1911). He established substantial diversity in quantitative features, which he termed 'phenotypical,' in genetically identical material, proving that genetics does not regulate all variation in observed traits. Because phenotypic variation within a genotype can hide phenotypic differences between genotypes, statistical analysis has been suggested for discovering genotype differences. This is where the word pheno- comes from. After 1950, the terms 'phenotyping' as a noun, 'to phenotype' as a verb, and 'phenome' as a collective noun were coined, which are now scientifically approved and commonly used in literature.

Forward and Reverse Plant Phenomics : The study of plant development, performance, and composition is known as plant phenomics. Forward phenomics use phenotyping techniques to discrimnate the useful genotypes with desirable features from a large pool of germplasm. As a result, the 'best of the best' germplasm line or plant variety can be identified. By screening a large number of plants at the seedling stage, high-throughput, completely automated, low resolution followed by higher-resolution screening approaches have hastened the plant breeding cycle. As a result, interesting traits can be recognized quickly at an early stage, and there is no need to grow plants in the field until they reach maturity. In forward phenomics, it is now possible to screen hundreds of plants in pots as they travel along a conveyor belt and through a room with automated imaging systems such as infrared or 3D cameras. The pots have barcodes or radio tags on them so that the system can tell which ones have plants with intriguing characteristics. The chosen plants can then be grown to yield seed, which can be used for further research and breeding. When the best of the best genotypes with desirable trait(s) are already known, reverse phenomics is applied. Traits that have been found to be useful in revealing mechanistic insight are now investigated in detail using reverse phenomics, and the uncovered mechanisms are then used in new techniques. As a result of reverse phenomics, we can learn about the mechanisms that make 'best' variations the best. A physiological trait can be reduced to biochemical or biophysical processes, and then to a gene or genes. For example, in the instance of drought tolerance, researchers are attempting to unravel the mechanisms underlying the trait and identify the gene or genes responsible. These genes can either be tested in germplasm or bred into new varieties.

Conventional phenotyping and Phenomics : Traditional phenotyping comes with a lot of bottlenecks in order to justify the experiments, which slows down the research process and is also time and labour intensive. The entire phenotyping of the bigger plot is rather tough, and even if it is completed, it may not be precise. Visual screening is used in classical phenotyping, although it is ineffective for physiological and biochemical characteristics. Destructive assay is used in some biochemical characterization. Because traditional phenotyping is prone to errors, exact justification for the experiment is challenging. This is why the science of phenotyping has evolved. For the refining and characterisation of a phenotype, phenomics is the systematic measurement and study of qualitative and quantitative features, including clinical, biochemical, and imaging approaches. Phenomics necessitates both "deep phenotyping," which entails collecting a large number of accurate and precise phenotypes, and "phenomic analysis," which entails determining the pattern and relationship between genotype-phenotype relationships and individuals with associated phenotypes.

High-throughput Phenotypic platforms used for phenotyping : A wide range of completely automated high-throughput phenotyping devices based on non-destructive measurements are available in numerous universities across the world (Furbank and Tester, 2011). HTP systems speed up phenotyping by utilising high-tech automated sensors, imaging devices, and computational resources (Furbank, 2009; Yang *et al*., 2020). Imaging techniques such as 3-D imaging, near infrared imaging, far infrared imaging, fluorescence imaging, visible light scanning, hyperspectral imaging, magnetic resonance imaging, and positron emission tomography are included in the HTP platforms, as well as the lemnatec technology phenocopter (Rahman *et al*., 2015). Clear imaging features, mechanical transport enabling the mobility of imaging plants, effective imaging formulas, and data analytics are all part of these systems (Gerke *et al*., 2009). These HTP platforms have evolved into sophisticated plant phenotyping equipment with sophisticated software systems (Paproki *et al*., 2012).

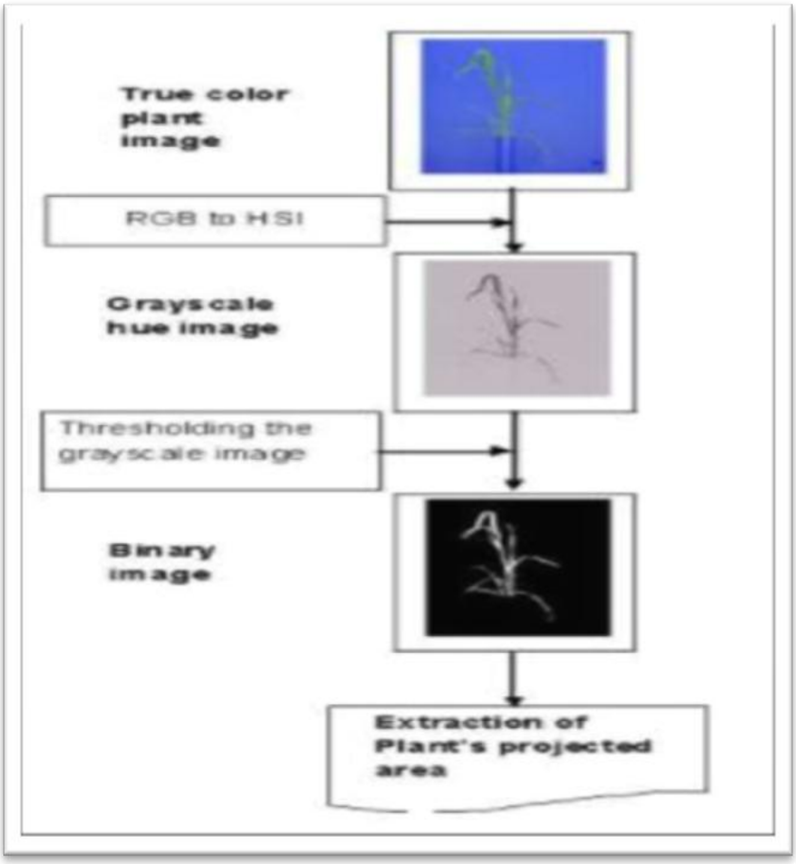

Fig.1: Hyperspectral imaging for phenotyping (Golzarain *et al.,* 2011)

Visible Light Imaging : Because of their low cost and ease of operation and maintenance, visible images have been widely used in plant research. Visible imaging is used to examine shoot biomass, yield traits, panicle traits, imbibition and germination rates, leaf morphology, seedling vigour, coleoptile length and biomass at anthesis, seed morphology, and root architecture in a controlled environment (in a growth chamber or a greenhouse). The projected leaf area in plants like *Arabidopsis thaliana* and maize is available through commercial systems based on visible imaging, which is one example for shoot biomass in a controlled environment. This approach extracted a mathematical relationship between these three visible images for the shoot biomass or leaf area using different viewing angles (typically two side views and a top view). The r2 value of the correlation between the digital estimation of the shoot biomass and the destructive harvest can be greater than 0.9. Leaf senescence can be measured by separating the yellow and green portions of the leaf after prolonged saltwater exposure, and this discovery can be linked to tissue tolerance to accumulated salt. These salinity tolerance components can be assessed on a single plant using visual image analysis. A series of standard image analysis pre processing and segmentation algorithms, such as the watershed algorithm, a colour segmentation method based on RGB space, and an image segmentation model, are used when using visible imaging extracts of plant growth morphology dynamics, root systems, or seed surface features. Additionally, new image processing methods have been discovered. De Vylderet al. 2012, for example, used colour segmentation based on phenotype parameters extracted from RGB space, such as shoot biomass (to quantify the image-based projected area), diameter (the maximum distance between two rosette pixels), stockiness, relative growth rate, and compactness (the ratio between the area of the rosette and the area enclosed by the convex hull of the rosette).

Fluorescence imaging: The artificial excitation of plant photosystems and study of the associated reactions can provide information about a plant's metabolic condition. Its fluorescence is the most relevant approach to describe. Fluorescence is light that is emitted when radiation of shorter wavelengths is absorbed. When the chloroplasts are exposed to blue or actinic light, some of the absorbed light is re-emitted by the chlorophyll, and the re-emission fraction of the absorbed light by the chlorophyll increases. The proportion of re-emission light vs irradiation varies and is determined by the plant's ability to metabolize the captured light. The fluorescence of the re-emitted light is an excellent indicator of the plant's ability to assimilate actinic light. Furthermore, by combining an actinic light source with brief saturating blue pulses, it is possible to evaluate the plant's photo assimilation efficiency, non-photochemical quenching, and other physiological plant parameters. UV (ultraviolet) irradiation (wavelengths range from 340–360 nm) produces two forms of fluorescence: red to far-red and blue to green, which is the basis for multicolor fluorescence imaging. By illumination with a single wavelength, this approach allows simultaneous collection of fluorescence emission from four spectral bands (blue (440 nm, F440), green (520 nm, F520), red (690 nm, F690), and far-red (740 nm, 740). The origins of blue and green fluorescence emission (with maxima near 440 and 520 nm) are cinnamic acids (primarily ferulic acid) found mostly in cell walls, while the origins of red and far-red fluorescence emission (with maxima near 690 and 740 nm) are chlorophyll molecules in the antenna and reaction center of photosynthetic photosystem II from chloroplasts in mesophyll cells. Fluorescence emission variations or, even more sensitively, changes in the relationship between distinct fluorescence ratios (F440/F690, F440/F735) can be utilised as stress indicators, and the F690/F735 ratio has been demonstrated to be a chloroplast content indicator.

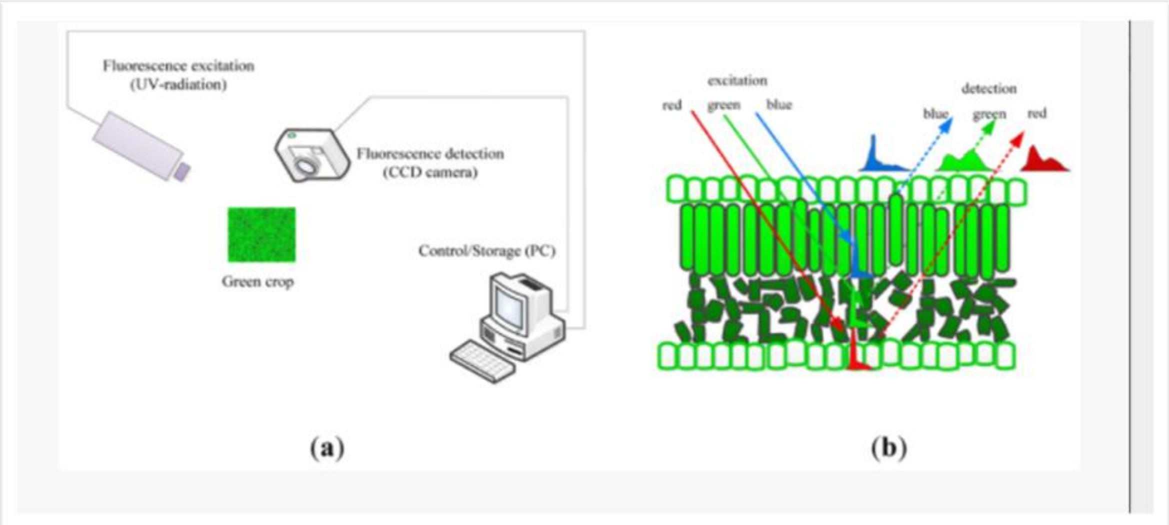

Fig. 2: Fluorescence imaging for high-throughput phenotyping (Lei *et al.*, 2014)

Thermal imaging: Infrared radiation can be visualised through thermal imaging, indicating the temperature of an object over its surface. Thermal cameras have a sensitive spectral range of 3-14 m, and the most popular wavelengths for thermal imaging are 3–5 m or 7–14 m. Therate of evaporation or transpiration from a leaf is a primary regulator of the leaf temperature, thermal imaging is used to monitor leaf surface temperatures to study plant water relations, and specifically for stomatal conductance. Reduced rates of photosynthesis and transpiration are common responses to biotic and abiotic challenges, and remote sensing of leaf temperature by thermal imaging can be a reliable technique to detect changes in the physiological status of plants in response to various biotic and abiotic pressures. Drought-prone environments have effectively exploited canopy temperature in breeding operations. Thermal imaging is used in plant phenotyping to detect changes in stomatal conductance, which is a measure of the plant's reaction to water status and transpiration rate, both in the field and in the greenhouse. Between anthesis and the blister stage, Romano *et al*. (2013) used thermal imaging at the canopy level in maize under reproductive stage drought stress. In maize water stress breeding programs, thermal imaging has been recognised as a viable method for phenotyping and screening. In Arabidopsis, thermal infrared imaging has also been proposed for use in the lab for mutant screens. Thermal infrared imaging was utilised to assess canopy temperature differences with surrounding air (for example, the canopy temperature depression, or CTD), and the results were employed as a selection criterion in drought resistant breeding programmes. Within-field variability for phenotyping site selection was identified using thermal images of a single cover crop under drought stress. In addition to oil and protein, thermal (NIR) imaging is used in seed kernels to determine the quantity of starch present in leaves (Jones *et al*., 2009). Rice and soybean germplasms have been tested for drought stress using NIR (Seelig *et al*., 2008). In wheat and barley, NIR assesses features linked with osmotic resistance under salt stress, (Lobete and colleagues, 2011).

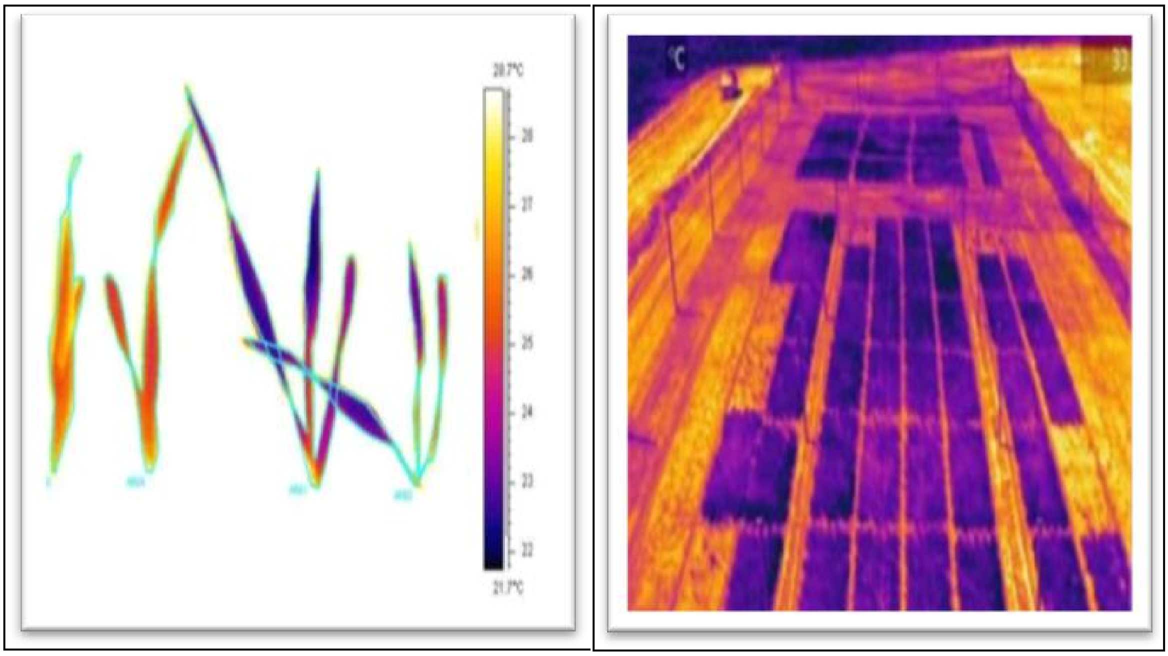

Fig. 3: Thermal imaging for high-throughput phenotyping (Furbank and Tester, 2011)

3D (Three-Dimensional) Imaging : A computer application combines many photos recorded from various angles using multiple cameras to create a 3D depiction of the plant (Weirman2010). Several measurements, such as leaf number, shape, angle, colour, leaf health, tiller number, shoot mass, and so on, can be made once a 3D image of the plant has been created. Light detection and ranging (LIDAR) techniques that scan a scene (Omasa *et al*. 2007) and stereo photography that uses two (or more) cameras are the two most prevalent methodologies for 3D imaging and mapping in plants (Biskup*et al*. 2007). The LIDAR approach produces an unconnected 3D point cloud that must be constructed from individual 3D space points to produce a 3D image, whereas 3D optical imaging data can be captured quickly but data processing is time intensive (Fiorani*et al*. 2012). To unravel essential areas of the rice genome affecting root architecture, (Topp *et al*.2013) used semi-automated 3D in vivo imaging and digital phenotyping for QTL mapping.

Hyperspectral Imaging : Hyperspectral imaging uses a continuous spectrum that spans the entire visible and near-infrared spectrum and is used for more detailed studies of pigmentation, hydration, biological compositions, and Vis. Spectral reflectance is the proportion of light reflected by non-transparent surfaces. Researchers utilise this spectral reflectance long before it can be seen to detect plants stressed by salty soil or drought (Bock *et al*., 2010). It has been used to measure leaf and canopy temperature in rice and panicle health status in wheat under heat and drought stress (Dale *et al*., 2013).

Magnetic resonance imaging : MRI uses a combination of magnetic fields and radio waves to create images. It's commonly used to image plant roots. MRI provides structural understanding about interior physiological processes by detecting nuclear resonance signals. MRI is utilised to visualise the symptoms caused by cyst nematodes (Hillnhutteret *al*., 2012). It's also been used to determine the water content of rice, wheat, and barley under stress. Many crops, including poplar, castor bean, tomato, and tobacco, have used it to provide realistic data on water diffusion and transportation in plants (Windt *et al*., 2006)

Phenocopter: Merz and Chapman developed the Phenocopter, a remote-controlled, gas-powered model helicopter, at CSIRO in 2011. Plant height, canopy cover, lodging, and temperature are measured. Fluorescence emissions as a function of water stress are investigated using hyperspectral and infrared cameras (Tejada *et al*., 2009). The use of a pilotless aircraft to capture foliage using thermal and narrowband multispectral remote sensing has been emonstrated (Berni*et al*., 2009).

Fig. 4: Phenocopter-A pilotless aircraft for remote sensing (Chapman *et al*., 2014)

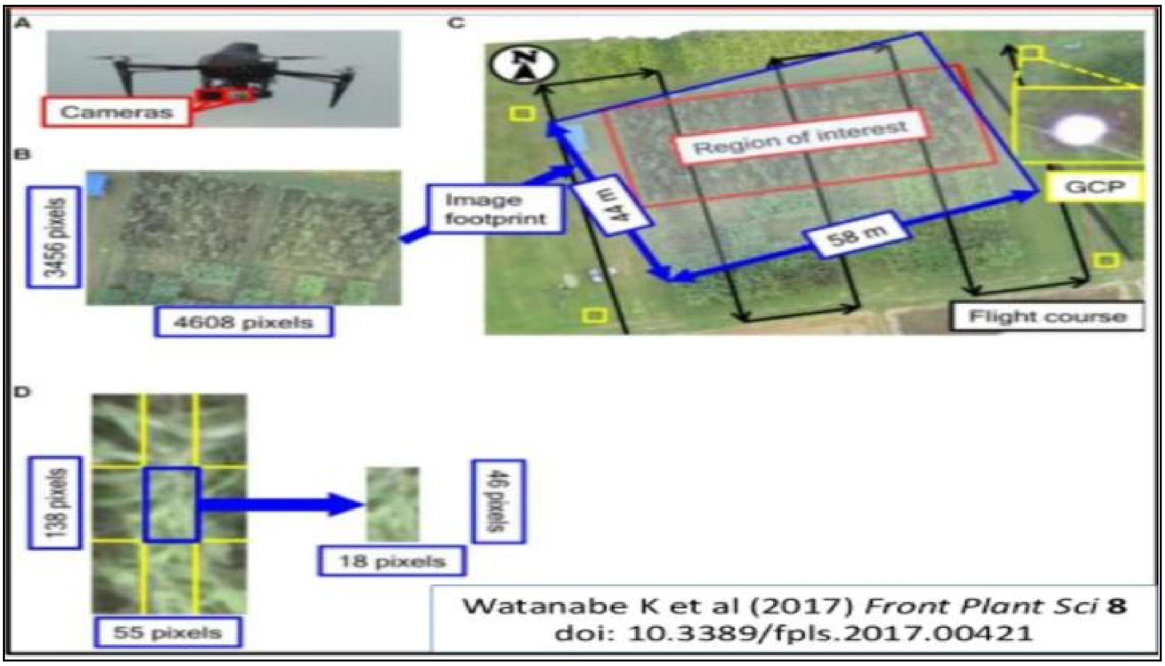

Fig. 5: high-throughput phenotyping techniques using hyperspectral imaging

Table 1: Emerging high-throughput phenotyping techniques and integrated sensor platforms applicable for plant trait assessment for field phenotyping

Sensor	Examples	Crop species	Trait/ phenotypic parameter	Applications	Advantages	Limitation	References
Hyper-spectral sensor	VNIR, SWIR	Rice, Wheat	Nitrogen status, nitrogenuse efficiency, water content, yield estimation, canopy components	Evaluate spectral properties, explore hyperspectral bands, estimate indices for fertilizer accumulated in plant organs, early detection of plant stress	Accurate estimation of nitrogen content and other biochemical or Physiological Status	Update model for new crop species, image processing is challenging, sensors are costly, large data Size	*Seiffert et al., 2010; Deery et al., 2014; Sadeghi-Tehran et al., 2021; Wang et al., 2021*
Thermal sensor	Thermal Infrared sensor, near-Infrared camera, FLIR Sensor	Wheat	Canopy temperature, drought tolerance, water use efficiency	Monitor crop temperature for abiotic stresses e.g., drought tolerance	Low-cost, precise and reliable in Repeated Experiments	Environmental factors have an impact on performance, very small temperature variations are undetectable, and cameras with higher resolution are heavier	*Costa et al., 2013; Deery et al., 2019; Sagan et al., 2019*
Visible light sensor	RGB sensor, visible light Camera	Rice	Shoot growth, phenology, greenness, plant vigour, leaf area	Visible phenotype parameters, classification of crop organs, greenness, growth and health, time series of vegetation indices	Affordable sensors are Available	Visual spectral bands and properties are limited, Changes in illumination conditions cause image blur and noise errors	*Kipp et al., 2014; Guo et al., 2015*
3D sensor	LIDAR Light Detection and Ranging) sensor, 3D laser scanner	Maize, Wheat	Plant height, canopy cover, above-ground biomass, crop architecture	Extract morphological traits of plants organs from 3D point clouds; measuring crop height and volume	3D plant information can be quickly Captured through close-Range Observation	LIDAR can be sensitive to small variations in path length, field applications can be challenging	*Müller-Linow et al., 2015; Guo et al., 2018; Jimenez-Berni et al., 2018; Qiu et al., 2019*

Continued table 1

Sensor	Examples	Crop species	Trait/ phenotypic parameter	Applications	Advantages	Limitation	References
Fluorescence sensor	Fluorescence camera, LIFT fluorometer	Wheat	Photosynthetic capacity, chlorophyll content, quantum yield	Measure photosynthesis, chlorophyll, water stress	Automatic and Rapid measurement of photosynthetic parameters	Limited for UAV imagery, can be affected by background noise, difficult to use in the field	*Chaerle and Van Der Straeten, 2000; Zendonadi dosSantos et al., 2021*
Multispectral sensor		Sorghum, Maize	Disease resistance, nutrient use efficiency, N content, biomass, grain yield	Multiple plant responses to nutrient deficiency, water stress, diseases, etc.,	High-resolution, fast	Sensors can be expensive, limited to a few spectral bands	*Zaman-Allah et al., 2015; Zhao et al., 2021*
Spectrometer		Maize	Water content, seed composition, yield	Leaf and canopy growth, disease evaluation, leaf area, chlorophyll content, canopy temperature, and crop responses	Handy and easy to use, Inexpensive	The quality of the data may be affected by soil background, spectral mixing could occur, and sensor calibration required	*Cozzolino, 2014; Andrianto et al., 2017; Chivasa et al., 2020; Cavaco et al., 2022*

Zhao et al., 2019

Unmanned Ariel Vehicle: The Further You Look, the More You See: Drones (also known as unmanned aerial vehicles or UAVs) provide a versatile platform for quickly collecting data across wide areas and potentially giving high spatial resolution photos (1 mm per pixel). Deep learning (DL) is an advanced IT technology that can handle millions of remote sensing photos with great accuracy and speed (Bauer *et al*., 2019). As a result, remote sensing has been widely utilized to track drought stress responses, measure nutritional status and growth, detect weeds and diseases, predict yield (Maes and Steppe, 2019), and discover QTLs (Maes and Steppe, 2019). (Wang *et al*., 2019). Canopy colour and texture features recorded at high spatial and temporal resolution by UAV platforms (Yue *et al*., 2019) aid phenotyping activities, as improved image quality and quantity provide detailed information for feature mining and analysis. However, there are some limitations to the utilization of UAVs that should be addressed: (1) the flight time and load capacity are limited; (2) local flight laws and regulations may be a constraint; (3) strict operating technician requirements should be implemented to ensure flight safety; (4) a low flight altitude will provide original images with higher quality but can also cause changes to the leaf architecture and physiology due to the strong wind produced by the UAV. Furthermore, lower flights take longer to fly, making it difficult to screen huge populations with low-altitude drone flights. As a result, appropriate flight altitudes and other camera settings related with various phenotyping goals should be researched further.

Aerial platforms					
Unmanned aerial vehicles (UAVs)	Broadly classified into Rotocopters, fixed wing systems, parachutes, and Blimps	Traits such as canopy cover, canopy height, crop lodging, growth indices, and canopy temperature can be estimated from the imagery	Rotocopters (i.e drones) can deploy a wide range of sensors, including thermal, multispectral, and hyperspectral cameras, high hovering capabilities, better flight time	speeds for image stitching, lens distortion, and overlap of the acquired images can affect orthomosaic, batteryuse and flying time may be limited Lower by the payload, and operability is limited in windy, wet, dull, variable light, or cold conditions	*Sankaran et al., 2015; Zaman-Allah et al., 2015; Chawade et al., 2019; Holman, 2020*
Satellite imaging	Digital Globe WorldView-2 satellite, WorldView-3 satellite, RADARSAT-2	In precision agriculture for germplasm evaluation, multi-location yield trials, field observation of crop biophysical parameters, weather predictions	Evaluation of moderate to large-sized trial, multi-location evaluation; provides automated coverage of isolated field trials across a larger geographical area	Affected by weather conditions, resolution, frequency of imaging, takes a long time from image acquisition to access, costly, higher frequency ofsatellite revisits, cloud cover can interfere with imaging	*Tattaris et al., 2016; Yang et al., 2017b; Yang, 2018*

Li *et al., 2014 and* Deery *et al., 2014*

Future Prospectus: Phenotyping has shown remarkable power to support fundamental crop research and crop breeding because to the rapid development of sensor techniques, machine vision, automation technology, fifth-generation mobile networks, cloud-based technologies, and artificial intelligence (DL). The construction of an International Crop Phenome Project (ICPP) should also be fostered in order to promote the next green revolution in crop breeding. To propose that multiomics international networks and varied fields collaborate to attain this goal, as well as coordinated financial support from several countries.

CONCLUSION

Plant breeders must first identify suitable genotypes with goal attributes by screening a collection of germplasm accessions in order to make successful genetic improvements in agricultural plants. After that, through hybridization, these target qualities are blended. Various approaches and methodologies for screening biotic, abiotic, physiological, and biochemical characteristics in agricultural plants have recently been established. In the digital age, these technologies have improved significantly. These advancements in plant phenomics are actually assisting in the refashioning of plant physiology. As a result of this multidisciplinary approach, important new advancements in plant science are expected. Phenomics allows researchers to investigate undiscovered areas of plant science, as well as to combine genetics and physiology to disclose the molecular genetic basis of a variety of previously untractable plant processes.

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