

High-throughput phenotyping in horticultural crops: Innovations, challenges, and the path ahead

Image-based phenotyping has become vital in the breeding, cultivation, and quality assessment of economically important crops. This article examines its advancements and applications in horticultural crops, highlighting imaging techniques such as RGB, thermal, hyperspectral, fluorescence, and tomographic imaging. These methods enable high-throughput phenotyping across various traits, including morphology, physiology, biochemistry, diseases, pests, and abiotic stresses. In addition to accelerating breeding cycles through rapid trait measurement, image-based phenotyping supports real-time monitoring and quick decision-making for activities like pesticide application, fertilization, and harvesting, ultimately improving yield and produce quality. It also plays a crucial role in postharvest phenotyping, ensuring quality during storage and handling. With its impact on both yield and product quality, image-based phenotyping is integral to the entire horticultural chain. The integration of machine learning and deep learning technologies is key to efficiently extracting valuable insights from the extensive data generated.

NON-invasive, image-based high-throughput phenotyping (HTP), coupled with advanced data analytics, is key to bridging the genotype-phenotype gap, accelerating genetic advancements in crop breeding. Deep phenotyping enables exploration of genetic factors underlying traits like stress tolerance and yield enhancement. By targeting traits of interest, HTP can reveal correlations between phenotypes and genetic markers, pinpointing quantitative trait loci (QTLs) or genes. Conventional phenotypic characterization methods, being manual and subjective, fall short in robustness, repeatability, and reliability over time. This 'phenotyping bottleneck' hinders understanding of phenotype-genotype correlations and environmental interactions. High-throughput phenotyping (HTP) typically involves capturing images to quantify traits throughout the crop growth cycle. A major advancement in high-throughput phenotyping is the ability to non-destructively capture plant traits, enabling time-series measurements to track growth and stress over time in individual plants. High-throughput image-based phenotyping refers to technologies that can image at least hundreds of plants each day. This scale enables the analysis of mutant populations, QTL detection, and the identification of gene-environment interactions. Thus, modern high-throughput phenotyping (HTP) is at the forefront of innovation, enabling automated extraction of phenotypic data from sensor inputs. These advancements leverage data analytics, machine learning (ML), and deep learning (DL), offering a transformative approach for long-term genetic gains in

crops. HTP significantly enhances selection accuracy and intensity, making it pivotal in advancing plant science.

Integrating high-dimensional HTP sensor data from advanced devices—such as RGB, near-infrared (NIR), infrared (IR), fluorescent, and hyperspectral cameras—with a systems biology, multi-omics approach holds significant potential to enhance crop resilience to climate challenges. In HTP, establishing efficient pipelines converts large volumes of image and sensor data into actionable insights through advanced data analysis and modeling, expediting the selection of crop varieties better suited for climate resilience. Addressing big data and image processing challenges is essential, as accurate phenotyping is critical for breeders aiming to rapidly develop improved crop varieties. This highlights the importance of adopting cutting-edge tools like computer vision, artificial intelligence (AI), machine learning (ML), and deep learning (DL).

Horticultural crops, primarily consumed fresh, are highly perishable due to their high water content, as seen in fruits and vegetables. The market value of these products relies heavily on both external (colour, shape, size, texture) and internal (soluble solid content, firmness) quality attributes, which evolve throughout maturity, ripening, and postharvest storage, necessitating regular monitoring. Currently, external quality is assessed through visual inspection, a slow and subjective process, while internal quality is measured using destructive lab tests or portable tools, which limit speed and sample size. Given the perishability and changing quality of horticultural



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products, image-based phenotyping offers faster, more accurate, and larger-scale postharvest monitoring. This enables immediate data analysis and faster crop management decisions, making image-based phenotyping vital for precise horticultural cultivation.

Phenomics concept

Phenomics is defined as the study of the phenome, which refers to all observable traits that a genotype or genome can display as a result of interactions between environmental factors and its developmental plan. The term 'phenome,' introduced by Davis in 1949, is rooted in Greek, where φαίνω means “to bring to light” and τύπος denotes shape or pattern. A phenotype consists of measurable characteristics (phenotypic traits) expressed by a genotype at a particular time, reflecting developmental stages and environmental conditions. These traits include physical (e.g., colour, shape), chemical (e.g., metabolites, pH), and biological (e.g., growth, physiology) attributes. Phenomics, like other 'omics,' explores this extensive range of traits, with High Throughput Phenotyping (HTP) enhancing efficiency through automation and imaging. Phenomics is the study of the phenome, or the full spectrum of possible phenotypes a genotype can exhibit influenced by both environmental factors and developmental programming.

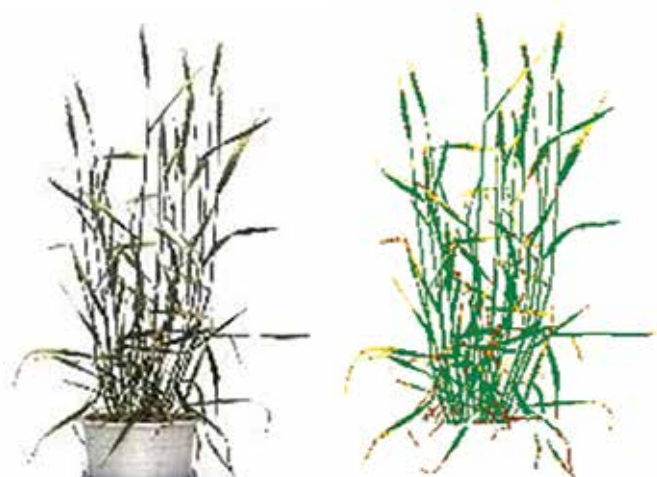
Available sensors for high-throughput phenotyping at NDPPC

The Indian Council of Agricultural Research (ICAR) in New Delhi has initiated the establishment of phenomics facilities across India. These facilities are located at ICAR-Indian Agricultural Research Institute in New Delhi, ICAR-Central Research Institute for Dryland Agriculture in Hyderabad, ICAR-Indian Institute of Horticultural Research in Bengaluru, and the National Institute of Abiotic Stress Management in Baramati, India. Specifically, ICAR-IARI in New Delhi has developed an advanced automated high-throughput plant phenomics facility, NDPPC, funded by the National Agricultural Science Fund (NASF), ICAR, New Delhi.

This facility is equipped with four cutting-edge climate-controlled greenhouses designed for cultivating plants under precisely defined environmental conditions. With 1,200 plant carriers embedded with RFID chip tags, the facility employs a moving field conveyor system to randomize plant positioning within the greenhouse,

facilitating automated weighing, watering, and imaging across multiple platforms. Furthermore, the facility features five automated weighing and watering stations, enabling precise induction of drought stress and measurement of transpiration and water-use efficiency. It incorporates several non-invasive image-based sensor platforms for assessing various plant traits

Visual high-resolution imaging: Utilizing high-resolution cameras positioned both above and alongside plants, reflectance within the visible spectrum (400-700 nm) is captured. This method enables measurement of various parameters including shoot/root growth, architecture, leaf greenness, leaf area, leaf rolling, senescence, growth rates, tillering, early vigour, plant height, phenology, biomass,

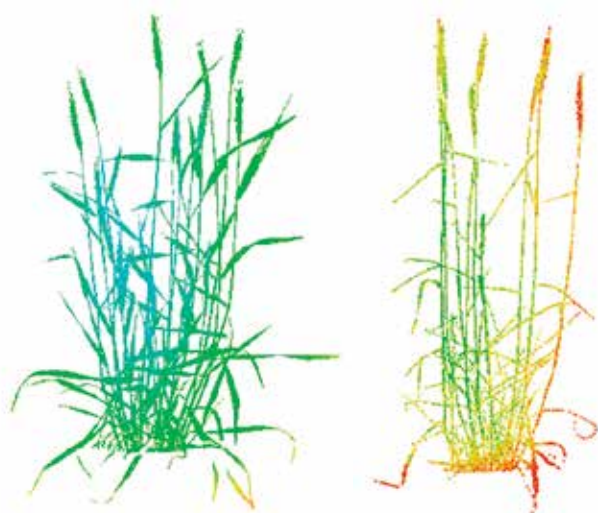


RGB visual imaging

convex hull, compactness, and eccentricity.

IR thermal imaging: Infrared energy (8 to 13 μm) emitted by plants is detected and converted into an electrical signal by an imaging sensor known as a microbolometer. This technology allows measurement of tissue temperature, which is primarily influenced by evapotranspiration. IR thermal imaging is valuable for inferring stomatal conductance and assessing plant health under biotic and abiotic stress conditions.

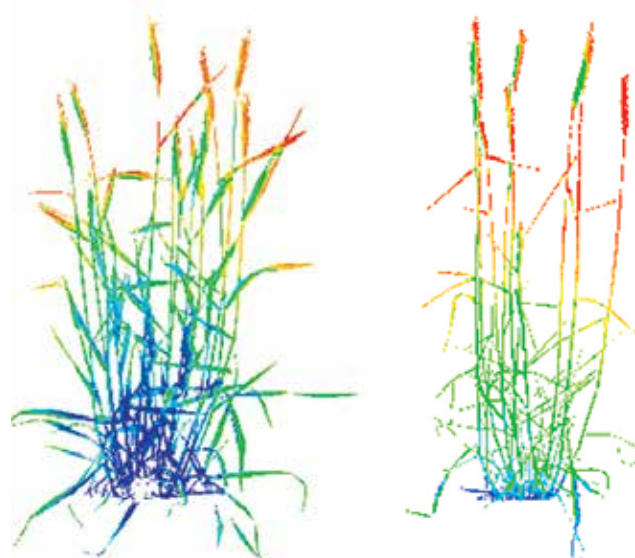
Chlorophyll fluorescence imaging: This technique exploits the emission of long-wave fluorescence by plant tissues following absorption of short-wave light, depending on the composition of molecules with inherent fluorescence properties. Chlorophyll molecules absorb light at short wavelengths and emit fluorescence at red/far-red wavelengths (680 and 735 nm). By measuring



Control

Drought

Thermal IR imaging

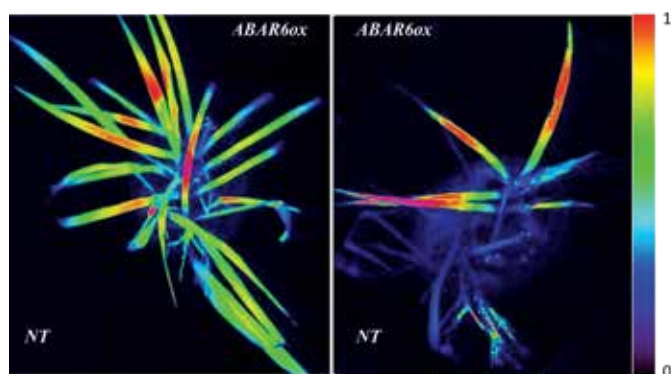


Control

Drought

Near-infrared (NIR) imaging

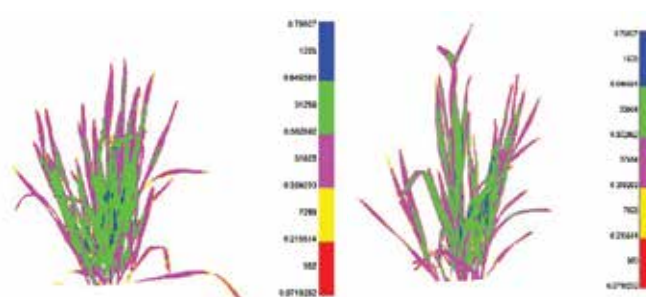
chlorophyll fluorescence, parameters such as maximum quantum efficiency of PSII, photochemical quenching, and non-photochemical quenching can be calculated. These parameters serve as sensitive indicators of resource



Control

Drought (-90 kPa)

Chlorophyll fluorescence imaging



Control

Drought

Hyperspectral imaging

availability and environmental stresses.

Near-infrared (NIR) imaging: The reflectance of plants within the 900 to 1700 nm range is influenced by water content. Plants exhibit significant reflectance in the 800 to 1400 nm range, while soil reflectance is minimal. NIR shoot imaging systems are employed to gauge water content and distribution within plants, as well as leaf thickness and leaf area index. Additionally, NIR root imaging systems are utilized to phenotype root soil moisture extraction patterns and root growth.

Visual-Near Infrared (VNIR) and Short-wave Infrared (SWIR) hyperspectral imaging systems: These systems capture spectral reflectance at nanometer resolution using VIS-NIR (400–1000 nm) and SWIR (1000–2500 nm) cameras. Various spectral indices are available to assess parameters such as chlorophyll content, relative water content, nutrient status, chemical composition, plant health, photochemical reflectance index, and genotype barcoding.

The automated weighing and watering stations will measure pot weights before and after watering to simulate stresses like drought, waterlogging, or nutrient

deficiency, with the goal of evaluating input efficiency and key physiological traits related to yield and stress tolerance across multiple plants at specific growth stages. This process is enabled by high-throughput phenomics platforms. The range of individual phenotypic traits and the spatio-temporal dynamics of phenotypic data generated in phenomics exceed those of traditional phenotyping methods, providing unparalleled depth and precision.

Unlocking genetic potential–Role of high-throughput phenotyping in horticulture

The role of high-throughput phenotyping (HTP) in modern plant breeding is pivotal, enabling efficient analysis of large plant populations and detailed trait acquisition. The adoption of HTP techniques and tools has revolutionized plant breeding by accelerating trait evaluation and selection processes. Here are several key functions of HTP in Horticulture:

Measurement of morphological traits: Morphological traits such as colour, size, shape, and texture are crucial for the appearance and visual assessment of horticultural produce. However, high-throughput phenotyping platforms are needed for faster, objective results. Image analysis is increasingly used to quantify these traits across crops. For example, grape berry colour, a key breeding trait, can be categorized into noir (red, blue, black) and non-noir (green, white) groups, which are easily distinguished

using RGB, Lab, and HSI colour spaces, revealing minor QTLs not detected by qualitative methods. Similarly, image-based analysis of strawberry fruit shape identified two QTLs related to the length-to-width ratio, while sweet potato shape features, like roundness and curvature, were used to predict marketability with 84.59% accuracy. In the food industry, where expert assessment is slow, image-based phenotyping provides rapid, reliable evaluations, such as quantifying apple slice browning and distinguishing between varieties. Colour, size, and texture are key indicators of maturity and ripening, with image analysis used to estimate these stages in fruits like plum and banana, significantly affecting market value. Hyperspectral imaging has also been explored for assessing these attributes in horticultural crops. Now-a-days, image-based fruit detection and counting and subsequent yield prediction is an emerging area and has been done using RGB sensors in apple, banana, grape etc.

Measurement of physiological traits: Physiological traits, such as photosynthesis and transpiration, reflect plant processes and indicate how plants function under various environmental conditions, responding to stresses and growth stages. These traits are measured using sensors like RGB, ChlF, multispectral/hyperspectral, and thermal imaging. Postharvest physiology in horticultural crops focuses on their responses during storage, handling, and processing, impacting ripening, shelf-life, and overall quality. The quality and shelf-life of horticultural crops are significantly influenced by storage and handling conditions. High-throughput postharvest phenotyping is essential for quickly and accurately assessing ripening, shelf-life, quality, safety, and biochemical content, enabling timely decisions to minimize economic losses. Traditional methods such as visual inspection, spectroscopy, and HPLC are labour-intensive, time-consuming, and often lack robustness, highlighting the need for efficient high-throughput techniques. Hyperspectral imaging has proven effective in detecting chilling injury, a common postharvest disorder, with over 91% accuracy in apple, peach, and kiwi fruit.

Biochemical component analysis: Horticultural crops contain valuable pigments such as anthocyanin, carotenoids, and chlorophyll, which are known for their antioxidant properties and health benefits. Traditional methods for quantifying these pigments involve labour-intensive lab extraction. While handheld devices offer nondestructive alternatives, they are not suitable for large-scale applications. Image-based phenotyping is gaining popularity as a non-destructive, rapid approach. Studies on red lettuce and cassava have demonstrated strong correlations between pigment content and image-derived indices. Additionally, optical sensors enable non-destructive monitoring of nutrient levels like nitrogen, phosphorus, and potassium, supporting more precise crop management.

Disease detection and quantification: Plant diseases are a major threat to global crop production, causing significant yield losses each year. Early and accurate detection

is essential for effective phytopathology and breeding efforts. Traditional methods, which rely on visual scoring and lab analysis, are time-consuming and subjective. Recently, rapid, high-throughput disease detection using image analysis has become more popular. This method is particularly important for horticultural crops, which are vulnerable to various pathogens before and after harvest. Image analysis allows for the detection and quantification of diseases such as apple scab, fire blight, powdery mildew, and bacterial blights, enabling timely intervention to reduce yield and quality losses.

Abiotic stress responses: Abiotic stresses such as drought, salinity, heat, and cold negatively impact plant growth and yield. Rapid and accurate phenotyping of plant responses to these stresses is essential for breeding resilient genotypes. Image-based high-throughput phenotyping plays a crucial role in efficiently screening breeding programs aimed at developing climate-resilient crops. Various imaging techniques have been used to assess horticultural plants' responses to different abiotic stresses. For example, hyperspectral imaging was employed to detect heat stress tolerance in ginseng, while cadmium stress in kale and basil was identified using high-throughput hyperspectral images, with the anthocyanin reflectance index proving effective across different stress levels.

Current status and Future perspectives

Publications on high-throughput phenotyping with image analysis are increasing, especially in agriculture and biological sciences, with 'fruit' being a dominant keyword, reflecting a surge in interest over the past five years. This trend highlights the growing focus on automating fruit trait measurement throughout growth, maturity, ripening, and postharvest phases. The choice of sensors in phenotyping depends on factors like platform, cost, and the trait being studied, with hyperspectral sensors being most common, followed by thermal sensors. A major challenge in image-based phenotyping is managing the vast data generated, with machine learning (ML) playing a key role in automating data analysis. ML algorithms, such as support vector machines, decision trees, and deep learning techniques like convolutional neural networks, efficiently process complex datasets and extract insights. Deep learning, in particular, excels with large datasets, enabling better processing of image-based phenotyping data. This approach, combined with advancements in sensing and data analysis, allows for the discovery of new traits, which, when integrated with omics data, can enhance crop improvement. However, challenges remain in storing and sharing large phenomic datasets, requiring standardized data sharing among researchers, academia, industry, and farmers. Reducing sensor and platform costs, along with automated big data analysis, will further boost phenomics' role in addressing global food demands.

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