

High throughput phenotyping approaches – New era of fruits improvement

The first step in the analysis of a trait of an organism is the determination of the type and/or the level of expression of the concerned trait; this is referred to as phenotyping. In general, accurate phenotyping is far more difficult than accurate genotyping for a variety of reasons, including the vast number of phenotypic traits (mostly in the case of fruits) and their sensitivity to the environmental factors. Many biological investigations, including mutant isolation, genomic selection, genome-wide association studies, and selection in plant breeding populations, require evaluation of thousands of lines/plants within a short period of time. Acquisition of sufficient relevant phenotype data of fruits/orchards are still challenging, especially for quantitative traits like tolerance to abiotic stress, polygenic disease resistance, and yield potential. Plant phenomics is described, as the study of plant growth, architecture, performance, and composition using high-throughput methods of data acquisition and analysis. The chief advantage of high-throughput phenomics approaches in fruits is the speed of data collection as field data that may take several days for acquisition by traditional approaches can be gathered in few hours using multiple sensors mounted onto a couple of vehicles (a field-based phenotyping platform). This would save time and allow multiple observations of a given fruit tree/Orchard in a single day. Phenomics is an area of intense ongoing research. The existing phenomics tools and techniques are being refined, their capabilities are being enhanced, and new approaches are being developed in different fruit crops.

ADVANCEMENT of fruit phenotyping by using high throughput imaging technology with sensors are emerging as new era of fruits improvement to speedup the phenotypic data collection. Thermal infrared images, which were merged with laser LIDAR data for 3D reconstruction, providing large-area 3D visual images in the Global Navigation Satellite System (GNSS) environment for multiple orchards. The NIR region reflectance can be measured/imaged and used for various analyses, including calculation of some useful indices like water index, normalized difference vegetative index (NDVI), etc. RGB-D (red, green, blue) camera to design image reconstruction that includes colour information and 3D shape information, and based on fruit colours and shapes. This algorithm is suitable for robot picking and can estimate the output yield of an orchard. Digital imaging in the visible wavelength (400–700 nm) is called visual imaging. This information allows quantitative measurement of growth, senescence, nutrient deficiencies, pathogen infections, and the consequences of stress-response mechanisms in different fruit crops. Hyperspectral imaging for the in-field estimation of grape berry soluble solids and anthocyanin concentration

could be phenotyped. All material which are above 0 degree Kelvin (-273°C), emit IR energy and the energy emitted from the measured object is converted into an electrical signal by the imaging sensor (microbolometer) in the camera and displayed on a monitor as a colour or monochrome thermal image. Magnetic resonance imaging (MRI) enables non destructive, high-resolution visualization of free water, which indicates frozen and unfrozen water in tissues and identification of healthy and frost-damaged tissues which is used to detect disorders involving water distribution, viz. watercore, core breakdown, chilling injury, bruising, decay, presence or feeding of insects.

Imaging technology for fruits

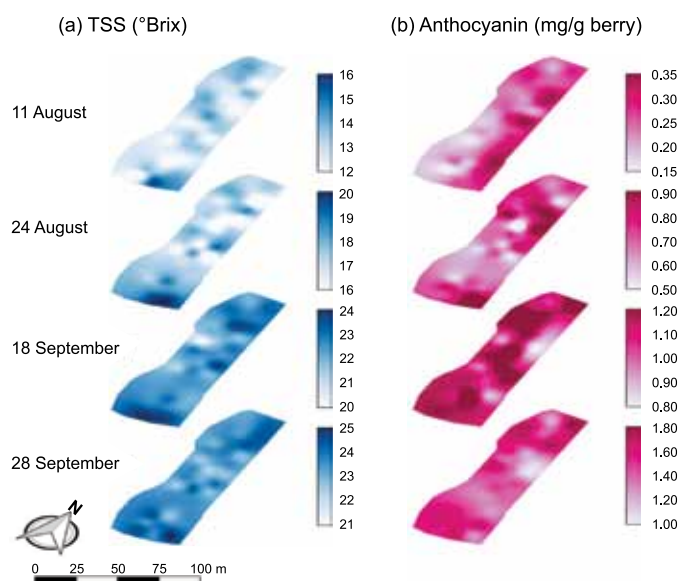
The images used for fruit phenotyping are acquired by one or more of the following high-resolution video cameras:

- Ultraviolet (UV) Florescence Imaging
- NIR hyperspectral camera (400–1000 nm)
- Visual (400-700 nm) Imaging
- Near-Infra Red (NIR) (700-1700 nm) Imaging
- VNIR, Hyperspectral (400-1000 nm) Imaging

- SWIR, Hyperspectral (1000-2500 nm) Imaging
- Infrared (IR) Thermal (8-16 μm) Imaging
- Chlorophyll Fluorescence (Photosynthetic efficiency) Imaging

The Visual (400- 700 nm) imaging is used for construction of growth profiles of the fruit tree by acquiring time series RGB images (400-700 nm of the electromagnetic spectrum). Image processing and segmentation occur either in the RGB space or in the HSV (hue, saturation, value) space. It provides 3D digital growth analysis with image from multiple angles to extract a mathematical relationship between these three digital images and biomass or leaf area. Shoot/Root growth and geometry, greenness, leaf area, senescence, root system architectures, growth rates, early vigour, biomass, nutrient deficiency, pathogen infection, stress response mechanisms of different fruit trees could be measured easily.

Hyperspectral imaging (HSI) is used to assess fruit composition mostly indoor under controlled conditions. This work evaluates a HSI technique to measure TSS and anthocyanin concentration in wine grapes nondestructively, in real time and in the vineyard. Hyperspectral images were acquired under natural illumination with a VIS-NIR hyperspectral camera (400–1000 nm) mounted on an all-terrain vehicle moving at 5 km/h in a commercial Tempranillo vineyard in La Rioja, Spain.



Prediction maps displaying the evolution of (a) TSS and (b) anthocyanin concentration during grape ripening.

Multi-sensor monitoring approaches in fruits

In a multi-sensor approach, images of the same fruit trees/orchards are captured using different sensors, e.g., visible and thermal sensors, or a combination of visible, thermal, and fluorescence sensors. During image analysis, the images from different sensors are laid over each other with the help of predetermined reference points within the concerned images. This greatly facilitates separation of the imaged object from the background materials. A combination of two or more imaging methods might generate more phenotypic information of greater reliability. The individual imaging techniques can reveal the symptoms of a wide range of stresses at an early stage, but the use of images from multiple sensors may permit the identification of the stress responsible for the observed symptoms. For example, leaf chlorophyll content decreases in response to both water stress and nitrogen deficiency; this change is readily detected by fluorescence imaging. However, water stress also leads to stomatal closure; this in turn leads to increased leaf/canopy temperature, which is easily detected by thermography. In contrast, nitrogen deficiency does not affect stomatal closure so that there is no change in leaf/canopy temperature. Therefore, a combination of fluorescence and thermal imaging would allow the determination of whether water stress or nitrogen deficiency is the real cause of the observed decrease in chlorophyll contents of the test plants. Clearly, the optimal combination of different sensors would depend on the physiological effects of the different stresses that are to be distinguished.

Washington State produces about 70% of total fresh market apples in the United States. One of the primary goals of apple breeding programs is the development of new cultivars resistant to devastating diseases such as fire blight. The overall objective of this study was to investigate high-throughput phenotyping techniques to evaluate fire blight disease symptoms in apple trees. In this regard, normalized stomatal conductance data acquired using a portable photosynthetic system, image data collected using RGB and multispectral cameras, and visible-near infrared spectral reflectance acquired using a hyperspectral sensing system, were independently evaluated to estimate the progression of fire blight infection in young apple trees. Sensors with ranging complexity – from simple RGB to multispectral imaging to hyperspectral system – were evaluated to select the most accurate technique for the assessment of fire blight disease symptoms. The features extracted from RGB images (e.g., maximum length of senesced leaves, area of senesced leaves, ratio between senesced and healthy leaf area) and multispectral images (e.g. vegetation indices) also demonstrated potential in evaluation of disease rating.

The average classification accuracy achieved using visible-near infrared spectral reflectance data during the classification of susceptible from symptomless groups ranged between 71 and 93% using partial least square regression and quadratic support vector machine. In addition, fire blight disease ratings were compared with normalized difference spectral indices (NDSIs) that were generated from visible-near infrared reflectance spectra. The selected spectral bands in the range 710–2,340 nm

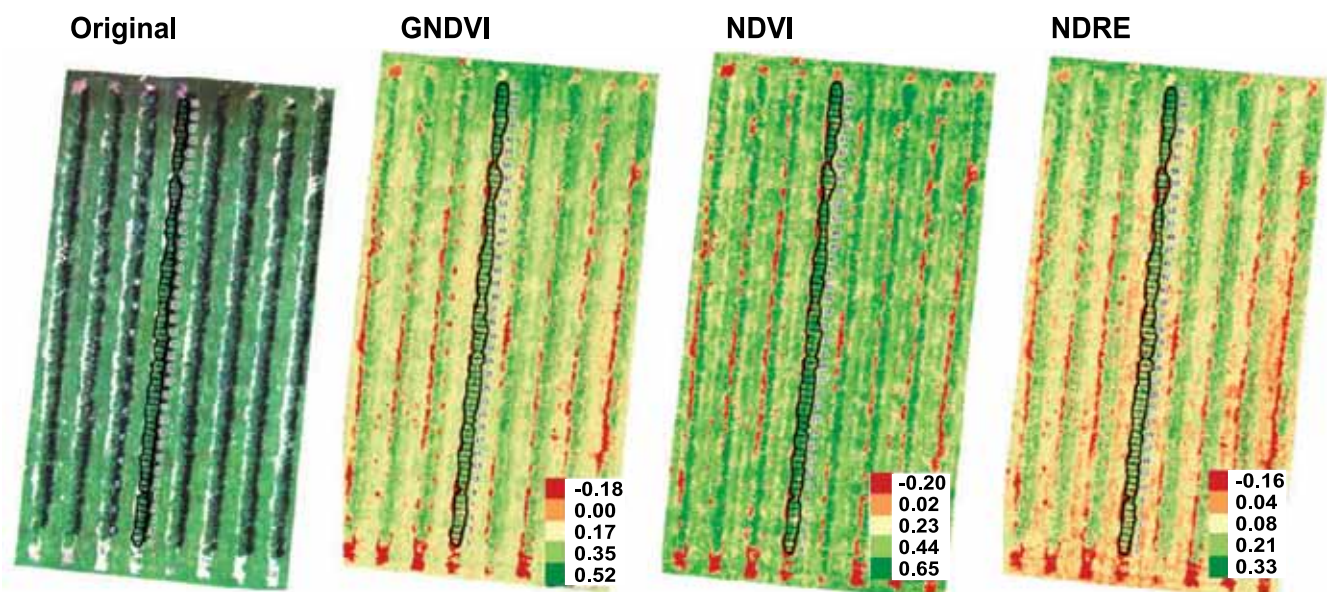


(a) Original RGB side-image with red, green, and blue lines in right side showing the relative gray scale intensities in the R, G, and B channels, respectively, of the yellow line highlighted in the image; (b) processed RGB image where the senesced leaves identified during image processing are marked in yellow; and (c) processed RGB image where the healthy leaves identified during image processing are marked in red. The total number of pixels representing senesced and healthy leaves, and maximum length of senesced leaf area were used for feature extraction. Source: Jarolmasjed *et al.* (2019).

used for computing NDSIs showed consistently higher correlation with disease severity rating than data acquired from RGB and multispectral imaging sensors across multiple seasons. In summary, these specific spectral bands can be used for evaluating fire blight disease severity in apple breeding programs and potentially as early fire blight disease detection tool to assist in

production systems.

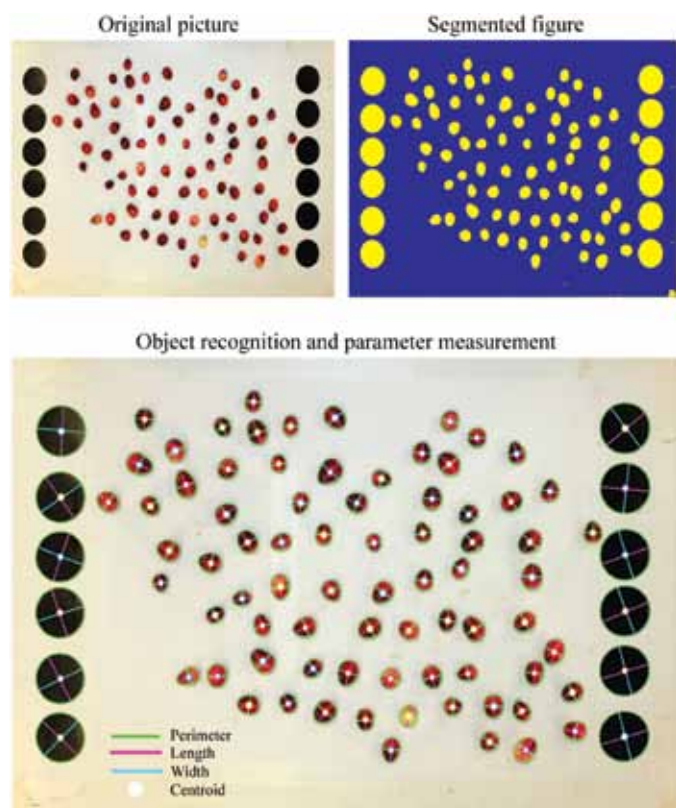
Given specific spectral bands could be used for evaluating fire blight disease severity in apple breeding programs. In addition, these indices also showed potential to be used as early disease detection tools that could assist in timely crop management in production systems.



The scales in GNDVI, NDVI, and NDRE represent the range of vegetation index data. The heterogeneity in tree canopy was a function of growth and disease status of each tree. The black vector superimposed on the original and vegetation index images represent segmentation of each tree. Source: Jarolmasjed *et al.* (2019).

GiNA, efficient and high-throughput software for fruit phenotyping

This is high throughput software for measuring horticultural traits such as shape- and colour-related parameters. Computations performed by GiNA for background extraction on cranberry fruit seeds are mentioned below:



Source: Garcia *et al.* (2016).

Magnetic resonance imaging (MRI) application in fruits

Magnetic resonance imaging (MRI) enables non-destructive, high-resolution visualization of the distribution of bound and free water, which indicates the spatial organization of organs and tissues. MR-Based measurable attributes are Proton density (Proton is the preferred nucleus for most imaging studies of fruits due to its high abundance, high magnetogyric ratio and sensitivity), Chemical shift (The change in the resonance frequency of the nucleus of interest to the resonance frequency of a reference nucleus measured at the same magnetic field and could provide information regarding the chemical constitution and hence are sensitive indicators of quality and maturity of fruits as the presence of specific chemicals), Diffusion constants (The diffusion constant of water within firm tissues is substantially different from that in liquid water or necrotic tissue).

Phenotyping facilities in India

A. Field Phenotyping facility

1. IARI, New Delhi
2. GKVK, UAS, Bengaluru
3. ICRISAT, Hyderabad

Apple	Storage time, internal browning (IB) and degree of tissue degradation	Defraeye <i>et al.</i> (2013)
	Disorders involving water distribution, watercore, core breakdown, chilling injury, bruising, decay, presence or feeding of insects	McCarthy <i>et al.</i> (1995) Wang <i>et al.</i> (1988)
Mango	Internal browning	Elizabeth and Roy (1993)
	Detection of internal defects such as bruising, chilling injury, and insect damage in Kensington Pride mango	Mazucco <i>et al.</i> (1993) Joyce <i>et al.</i> (1993), Joyce <i>et al.</i> (2002)
Pears	Growth stage Core breakdown Softness/firmness	Wang and Wang (1989), Lammertyn <i>et al.</i> (2003), Zhou and Li. (2007) Kimura <i>et al.</i> (2011); Geya <i>et al.</i> (2013)
Grapes	Organic acids and eight amino acids, internal characteristics of berries and degrees Brix distribution	Andaur <i>et al.</i> (2004)

Source: Srivastava *et al.* (2018).

B. Controlled Phenotyping facility

1. IARI, New Delhi
2. NIASM, Malegaon
3. CRIDA, Hyderabad
4. IIHR, Bengaluru

Future directions of high throughput phenotyping for fruits improvement

Phenomics involves both extensive and intensive phenotyping. In extensive phenotyping, a large number of phenotypes are evaluated in a limited number of contexts, e.g., analysis of the expression of all genes in a single tissue at one stage of development. But in intensive phenotyping, one or few phenotypes are characterized in a great detail, e.g., monitoring of the expression of one gene in all the tissues over the various developmental stages. Further, the phenomics data should be collected from an orchard exposed to a large number of different environments. Therefore, phenomics studies will always involve prioritization of the phenotypes to be investigated and the environments in which they are to be evaluated. The phenotype data can be used to build a database that may ultimately allow linking of gene sequences to different fruit tree canopy and function. The data for the above database will have to be measured under clearly defined environmental conditions, described objectively in mathematical terms, and stored in a digitized and easily searchable format. The future for the use of chlorophyll fluorescence in postharvest applications looks quite bright. At the moment, the technique shows the greatest potential in the areas of quality control (using a portable system) and development of feedback control equipment for atmospheric modification in CA storage (using a newly developed instrumentation). However, the use of imaging equipment for fluorescence pattern analysis on fruit or vegetable surfaces may provide great potential as a tool for

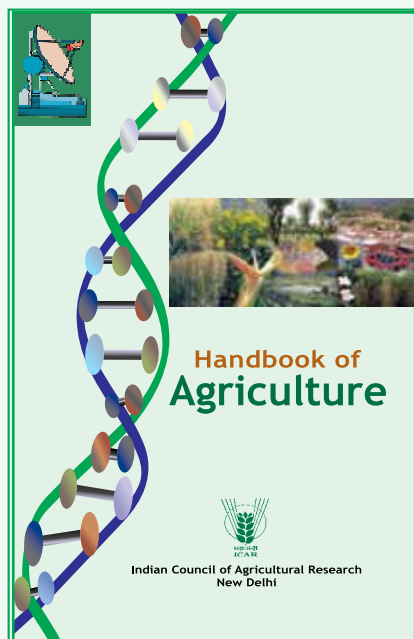
on-line sorting systems. Despite the advances made in the use of chlorophyll fluorescence in postharvest applications, it is quite clear that much more research is required to make the technology a practical commercial tool. It is important that these studies also adopt the intensive approach. This change would necessitate considerable enhancement in the capabilities, including the throughput of the phenotyping techniques coupled with a large reduction in their costs. Such phenomic data, of necessity, will comprise a very large number of different phenotypes (p) scored on a large number of individuals (N), but the p will always be much larger than N. There is an urgent need to develop suitable statistical models for analysis

of such data since the available methods are not entirely satisfactory. It will be highly desirable that the software developed for the statistical analysis of the phenotypic data permit automated data analysis in different fruit crops.

For further interaction please write to:

Drs Kripa Shankar, Lobsang Wangchu, Senjam Romen Singh, Barun Singh, Nongthombam Devachandra, Thejangulie Angami, Central Agricultural University, Pasighat, Arunachal Pradesh 791 102. *Corresponding author
E-mail: rawkripa.s99@gmail.com

Handbook of Agriculture



The Handbook of Agriculture is one of the most popular publication of the ICAR with a wider readership. The present edition presents science-led developments in Indian agriculture, the ongoing research efforts at the national level and with some ideas on the shape of future agriculture. While information in some chapters such as Soil and water, Land utilization, field and forage crops has been updated with latest developments, many new topics such as the Environment, agrobiodiversity, Resource conservation technologies, IPM, Pesticides residues, Seed production technologies, Energy in agriculture, informatics, Biotechnology, Intellectual Property Rights, Agricultural marketing and trading and Indigenous Technical Knowledge have been included in the present edition. For those who take intelligent interest in agriculture – and their number is increasing fast – the present edition would serve as a useful book.

TECHNICAL SPECIFICATIONS

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Directorate of Information and Publications of Agriculture
Krishi Anusandhan Bhavan, Pusa, New Delhi 110 012
Tel: 011-25843657, Fax: 09-11-25841282; E-mail: bmicar@gmail.com