The Pharma Innovation Journal 2020; 9(8): 184-191

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(8): 184-191

www.thepharmajournal.com
Received: 04-06-2020
Accepted: 06-07-2020

T Ilakiya
Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu, India

E Parameswari
Department of Environmental Science, TNAU, Coimbatore, Tamil Nadu, India

V Davamani
Department of Environmental Science, TNAU, Coimbatore, Tamil Nadu, India

Dumpala Swetha
Department of Vegetable Science, Dr. YSR Horticulture University, India

E Prakash
Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu, India

Corresponding Author:
T Ilakiya
Department of Vegetable Science, TNAU, Coimbatore, Tamil Nadu, India

High-throughput crop phenotyping in vegetable crops

T Ilakiya, E Parameswari, V Davamani, Dumpala Swetha and E Prakash

DOI: https://doi.org/10.22271/tpi.2020.v9.i8c.5035

Abstract
In the era of climate change with increasing global populace, there is a requisite for developing high yielding climate resilient varieties in a shorter span. Integrating the modern techniques of high-throughput phenomics with the gains of genomics would lead to increase in the efficiency of breeding techniques for the rapid development of such varieties. The development of plant phenomics facilities in vegetable breeding have been developing in recent years. High-throughput plant phenotyping involves the use of various imaging techniques, where the images are collected and are then analyzed in some specialized software's. Apart from getting the phenotype traits of the plant these techniques are used to analyze different biotic and abiotic stress traits as well as the nutrient status of vegetable crops. The high-throughput plant phenotyping platforms would help the vegetable breeder in saving their time, as the conventional phenotyping is a time-consuming process. HTTPs delivers more reliable and accurate results. It would also help to find more pertinent solutions for the serious issues that limits the vegetable crop production.

Keywords: Phenotyping, vegetable, imaging

Introduction
The greatest future challenge in plant science is the global food security, as it is estimated that population could reach more than 9 billion by the year of 2050. This goal is a challenging one for breeders since the expected increase in the yield should be of 2.4% rather it is only 1.3% as of now (Ray *et al.*, 2013) [52]. Over the past 50 years, the extensive agronomic and breeding strategies are most appreciable for increasing crop yield, new varieties release, adoption of some new method and technology in irrigation, pesticides, synthetic fertilizers etc. yet it is not sufficient to meet the demand. Climate change that are due to natural and anthropogenic activities have been tremendously intensified and are unpredictable in future. Drought, uneven and intense rainfall pattern, high or low temperature and other stresses that are occurring as a result of climate change pose a high risk on quality and quantity of the food produce. Vegetables known as protective food has an immense quantity of nutrients comparing to that of cereals. Due to their annual nature and their quality, vegetables form a major part of food production and security. Hence there arises a need for the development of climate re-silent vegetable varieties (Tripodi *et al.*, 2018) [61].
In the era of advancement with biotechnological tools such as marker assisted selection, marker assisted recurrent selection, marker assisted backcrossing and various transgenic te-chnologies, improved varieties with tolerance to biotic and abiotic stresses can be easily developed compared to that of the conventional traditional plant breeding programs. In past few decades the evolution of genomics has generated a massive impact towards crop breeding. The cost involved in genome sequencing have been drastically reduced and scientist are now been able to sequence ample amount of genotype for allele mining and association mapping (Jackson *et al.* 2011) [27]. But there is a bottleneck in linking physiological and phenotype data to sequenced genome data, hampering the use of genomic techniques. Manual plant phenotyping is a labour intensive and time-consuming process. Hence a need arises for the high-throughput and non-destructive evaluation of crop phenotype. Now-a-days high-throughput crop phenotyping platforms integrating the field such as plant biology, engineering, mathematics and computer sciences are been slowly developing, helping to break the bottleneck for understanding the genotype and phenotype interaction (Zhang *et al.*, 2019) [76].

Phenomics
In 1909, Wilhelm Johannsen introduced the term genotype and phenotype. An individual's genotype indicates all its genetic material, whereas the phenotype comprises any observable

trait or a character. The term phenome indicates whole plant phenotype (Fig. 1) and can also be referred as the genome expression in a particular environment. Additionally, the phenotype encompasses some set of characters that are observed either visually or with some specialized analytical tools and describe as the interaction between genotype and environment. Steven A Garan coined the term phenomics. As a whole phenomics is the wide scale acquisition of multidimensional phenotypic data of an organism (Houle *et al.,* 2010) [25]. Phenomics is further inter-related to other omics technologies such as transcriptomics, genomics, fluxomics or metabolomics to analyze the performance of a plant in the field further linking it to core molecular genetics.

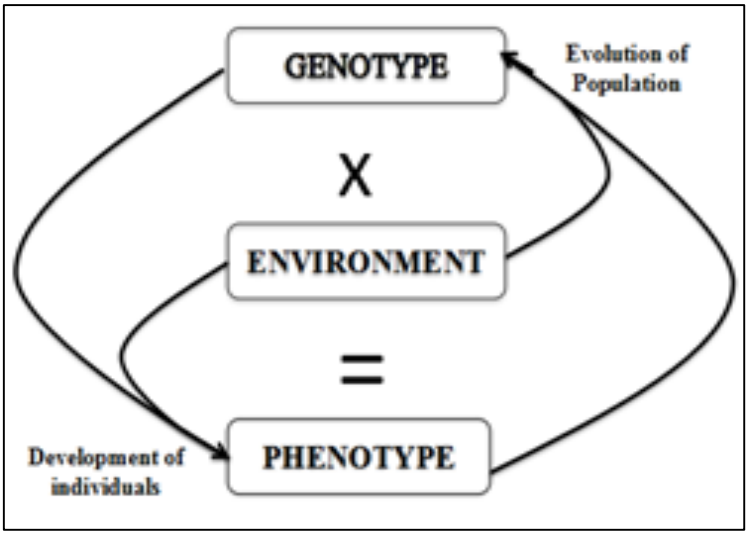

Fig 1: Phenotype

The plant phenomics can be performed in the plants that are grown both in controlled (greenhouse) condition or at field (Fig 2). Here in controlled conditions either the plants can be moved to the sensors or sensors can be moved to the plants whereas in the field condition only sensors can be moved to the plants. Phenomics are of two types *i.e.,* forward and reverse phenomics (Rahman *et al.,* 2015) [49]. In forward phenomics, phenotyping is done for large number of plant population that helps to identify the trait/plant that are suitable for a particular situation. In reverse phenomics, the desired trait or phenotype that is suitable in a population is known already and the researchers later try to find the mechanism underlying with the specific character that allows them to exploit the candidate genes that are associated with the character and can be further introgressed to obtain new varieties (Furbank and Tester, 2011) [19].

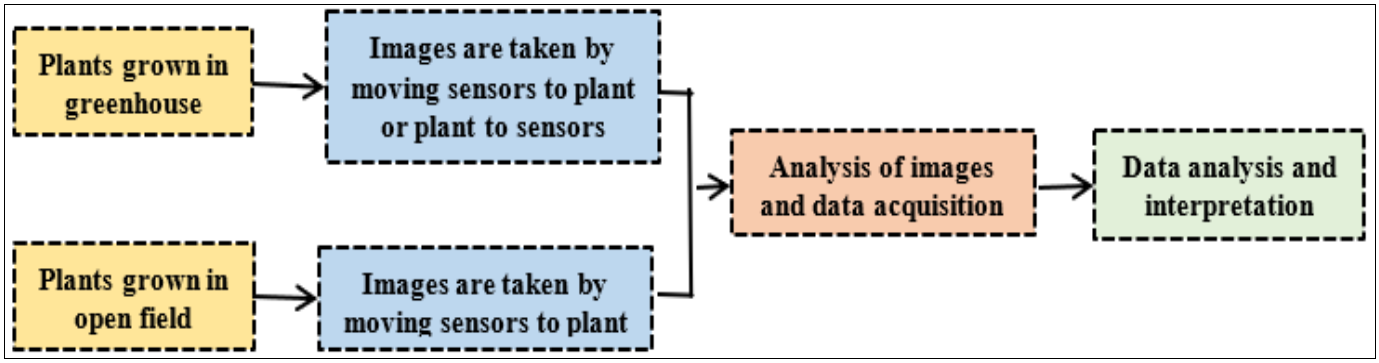

Fig 2: Steps involved in plant phenotyping

Need For High Throughput Phenotyping

- To replace the outdated phenotyping tools
- To accelerate the genomic technologies
- For deriving a new trait that were not considered before
- To study the phenotype-genotype map
- To phenotype whole population in a short period
- For dynamic phenotyping
- To increase accuracy as automation and robotics techniques are involved
- For a non-destructive phenotyping

Imaging Techniques in Plant Phenotyping

HTTPs (High-throughput Plant Phenotyping Platforms) in vegetable crops (Table 1) have been developing in recent years with new imaging technologies, sensors, automation and robotics. Based upon the designs used, either the sensor moves to the plant or the plant moves to the sensors. It generally employs screening of huge population to observe the presence of genetic variation for the given trait. Growth condition of the plants are well defined and strictly monitored. Phenotypic data that were precisely collected were subjected to further analysis to examine the relationship between the plant phenotype and genotype for the trait. The imaging technologies (Fig 3) used in HTTPs are detailed below:

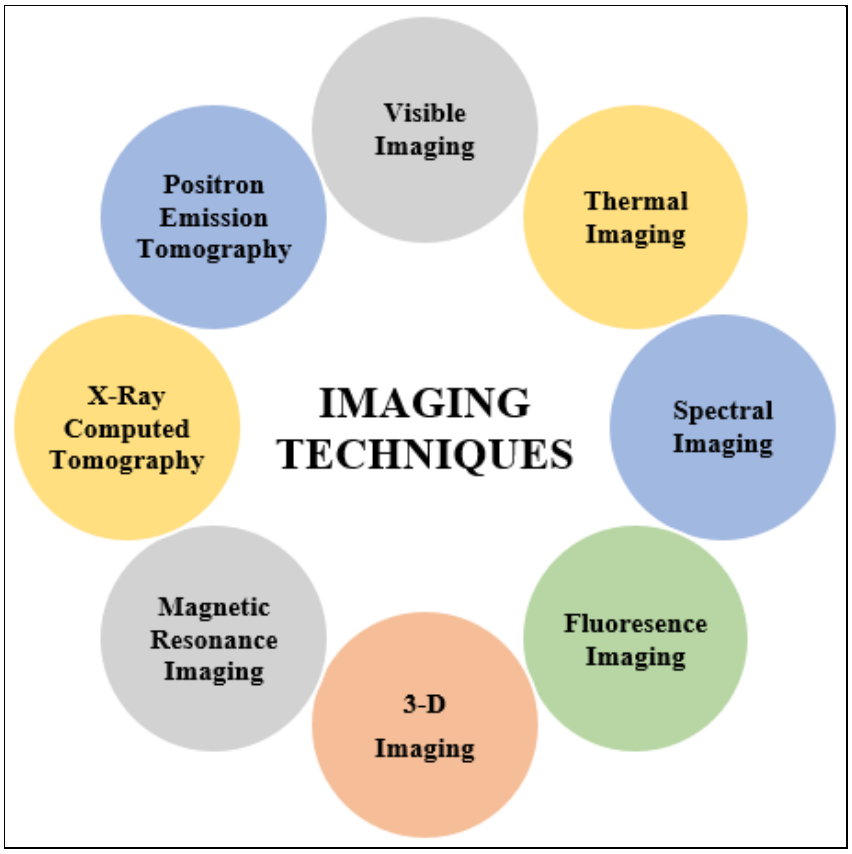

Fig 3: Imaging techniques used in HTTPs

1. Visual imaging

The visual imaging technology uses a simple RGB (red-green-blue) cameras to capture the plant images, this usually mimics a human eye. The common most application of visible image depend on silicon sensors (CMOS or CCD arrays) sensitive to visible light band lying in a wavelength of 400-700 nm and captures images in two dimensions for further analysis. They are broadly used for imaging plant structure for its ease of maintenance and low cost. The traits that can be measured using visual imaging includes shoot biomass, yield traits, biomass at anthesis, germination and imbibition rates, coleoptile length, seedling morphology, seedling vigour, leaf area, leaf morphology and root architecture (Kumar *et al.*, 2015) [31]. Removing the shadow of the canopy, only small differences in brightness and colour between the background and leaf and influence of light for the automatic image processing are the few limitations in working visual imaging technology that must be viewed to minimize the error that occurs.

2. Fluorescence Imaging

When the plant absorbs the radiation of shorter wavelength it emits a light termed as fluorescence. It occurs when the plant/compound absorbs light of a wavelength and emits light of a different wavelength. In the plant the typical part of fluorescence is the chlorophyll complex (Lee *et al.*, 2010) [33]. During fluorescence, there is a re-emission of a part of a light observed by the chlorophyll, when there is an irradiation of actinic or blue light in the chloroplast. There is a positive correlation between the light that is re-emitted in the radiation absorbed and plant's ability to metabolize the light that is harvested. Fluorescence imaging system includes the fluorescent signal excitation (optical transmission component and excitation light source), the fluorescence signal collection component and an amplification and signal detection system (Gorbe and calatayud, 2012) [9]. The cameras used in fluorescence imagining system have some specific pulse light and spectral cut off filters to measure the dynamics of fluorescence emission, making it sensitive for a particular spectral region at which fluorescent signals were emitted. Fluorescence imaging flashes a blue light (less than 500 nm) upon the plants, which later emits the fluorescence light with a range of 600 nm to 750 nm. The fluorescence is later photographed and were converted into some false signals by specific computer program allowing the scientist to observe the variance in fluorescence. Fluorescence imagining are used to characterize photosynthetic activity and plant health related to pathological and physiological traits. They are used to access the plant respiration function to detect the effect of plant disease and insect resistance genes and to monitor the plant pathogen. Additionally, they are used to diagnose the plant response to biotic and abiotic stresses such as salinity and drought. But for analyzing the whole canopy or plant, advanced imaging technology such as LIFT (Laser induced Fluorescence Transients) and sun - induced fluorescence are used currently (Rascher and Pieruschka, 2008 and Meroni *et al..*, 2009) [50, 39].

3. Thermal Imaging

Thermal imaging technique allows the visualization of the infrared radiation, giving an indication for the distribution of temperature across the surface of an object. The sensitive spectral thermal cameras ranges between 3µm - 14µm and the common most waveband used for thermal imaging are 3µm - 5µm or 7µm - 14µm (Zhang and Zhang, 2018) [75]. Infrared radiation transmission through the atmosphere within these two wave bands are close to its maximum value. The 3µm-5µm band has higher thermal sensitivity than 7µm-14µm

band as, the shorter wavelength corresponds to the higher energy level. Plants thermal measurement relies mostly on evaporation, with low and high temperature level reflecting opening and closing of stomata respectively. They are used in the detection of pathogen and to monitor the genetic variation that occurs.

4. Infrared and spectral imaging

Mostly, all object emits infrared radiation due to the internal molecular movement taking place. Infrared imagining is done at two specific wavelength range, one at 0.9µm-1.5µm called NIR (near-infrared) and other at 7.5µm-13.5µm called FIR (far-infrared). The near infrared cameras are used to study the water content and their movement in soil and leaves whereas, the far-infrared are used for studying the temperature. The plants that are subjected to NIR imaging are grown in clear container for taking their root measurement. NIR images are used for calculating the water absorption rate and their usage in plants. Additionally, the carbohydrate content in leaves, protein, oil and starch content in seed can also be measured (Cook *et al.*, 2012) [14]. Far-infrared imaging were used to measure and calculate the temperature difference within or among the canopy (Sirault *et al.*, 2009) [56]. They are also used to measure and calculate the stomatal conductance helping us to know the photosynthesis rate.

5. 3 D Imaging

3-D images are obtained by combining several images captured from different angles by various camera using some computer program. Once when a 3-D image of a plant are generated several measurements such as leaf shape, number, colour, angle, shoot biomass etc. can be recorded. Two approaches *i.e.*, LiDAR (Light Detection and Ranging) and stereo photography are used for 3-D imaging (Li *et al.*, 2014) [34]. LiDAR is one of the remote sensing technologies that are used to measure the target distance by illuminating the pulsed laser light to the target and later measuring the pulses that are reflected back. LiDAR creates a 3-D image during entire crop period and help to acquire a multi-source phenotypic data. Stereo vision uses two (or more) cameras to study the 3-D structures and motions. It is a vital subject in computer vision field for reconstructing three-dimensional scene geometry.

6. Magnetic Resonance Imaging

MRI employs the nuclear magnetic resonance for generating images and could detect the nuclear resonance signals that were originated from ^{13}C,^{1}H,^{15}N and ^{14}N. MRI combines both the radio waves and magnetic field to take the images and were commonly applied for imaging the plant roots. MRI has provided a solution for analyzing whole plant (Van and Van, 2013) [62], the distribution and quantification of water in the plant and to various organs non-destructively. MRI can analyze the root architecture of the plants that are in pots containing the soil mixture whereas previously the plants were grown in clear transparent agar. Besides, MRI are used to visualize the cyst nematode symptoms in sugar beet and bean root nodulation.

7. Positron Emission Tomography (PET)

PET is one among the nuclear imaging technique that can produce 3D picture or image of the functional process. PET detects the pair of gamma rays emitted indirectly from positron emitting radio-nuclei. The distribution of labeled compounds including ^{52}Fe, ^{13}N or ^{11}C are non-invasively imaged. During photosynthesis when CO2 are consumed, a 3D image of ^{11}C-labeled photo-assimilates transport are generated by PET. It also helps n studying the metabolism in plants. PET in combination with MRI, can provide the functional and structural traits and are used in independent analyzing of transport of water and labeled compounds (Jahnke *et al.*, 2009) [28]

8. X-Ray Computed Tomography (X-Ray CT)

This technology uses a computer processed X-ray for producing tomographic images of a particular areas of a scanned object and could produce a 3D image of an objects inside using a series of 2D radio-graphic images that were taken around in single axis of rotation. This technique could generate volumetric data of several structure with various densities including plant structure, root architecture and soil structural heterogeneity (Pierret *et al.*, 2002 and Stuppy *et al.*, 2003) [44, 57].

Table 1: High Throughput plant phenotyping studies carried out in vegetable crops using portable devices

Type of Analysis	Plant Species	Traits	Instrument	Reference
Visible spectrum/ Near Infrared	Tomato	Qualitative	LabSpec 5000	Ecarnot *et al*., 2013 [18]
		Antioxidants	HandHeld 2™	Szuvandzsiev *et al*., 2014 [58]
		Lycopene and physicochemical parameters	Varian Cary 500	Clément *et al*., 2008 [13]
		Varietal discrimination	USB2000 spectrometer	Xu *et al*., 2009 [70]
		Transgenic lines discrimination	FT-NIR spectrometer	Xie *et al*., 2007 [68]
		Harvest time	AgroSpec , VIS - NIR spectrophotometer	Yang, 2011 [70]
Chlorophyll fluorescence	Tomato	Drought stress	Handy FluorCam FC 1000-H system	Mishra *et al*., 2012 [41]
	Chicory	Cold stress	CF Imager	Devacht *et al*., 2011 [16, 36] and Lootens *et al*., 2011 [36]
	Bean	*Pseudomonas syringae* infection	Fluorcam	Rodriguez-Moreno *et al*., 2008 [54]
	Melon	*Dickeya dadantii* infection	FluorCam 700MF	Pineda *et al*., 2018 [45]
	Cabbage	Seedling leaf spots	Hitachi F-4500 fluorescence spectrophotometer	Chiu *et al*., 2015 [11]
Chlorophyll fluorescence and	Bean	Botrytis infection, magnesium deficiency	Homemade built	Chaerle *et al*., 2007 [10]

Thermo Imaging				
NIR Hyperspectral imaging	Tomato and Eggplant	*Alternaria solani* and *Epilachna vigintioctopunctata*	ASD FieldSpec Pro FR spectrometer	Apan *et al*., 2005 [2]
	Tomato	*Rhizopus stolonifer* spores	Homemade built	Hahn, 2004 [22]
		Leaves damaged by leaf miner	Nexus FT-NIR spectrometer	Xu *et al*., 2007 [68]
		Surface defects detection	ImSpector V10	Xing *et al*., 2006 [67]
		Ripeness	ImSpector V9	Polder *et al*., 2000 [46]
	Leafy vegetables	Chlorophyll content	ASD Fieldspec FR spectroradiometer	Xue and Yang, 2009 [70]
	Spinach	Quality during storage	EMCCD Luca-R camera-Hyperspec® VNIR	Diezma *et al*., 2013 [17]
		Crop canopy under water	Specim V10 spectrometer	Corti *et al*., 2017 [15]
	Water melon	Lycopene, Carotene, and TSS	NIR On-Line® X-One	Tamburini *et al*., 2017 [59]
	Spinach	Nitrate distribution	Compovision™	Yang *et al*., 2017 [72]
		Freshness of leaves	ImSpector	Zhu *et al*., 2019 [77]
Hyperspectral and Fluorecence imaging	Lettuce	Plant traits under extreme temperature and salinity stress treatments	Series VNIR Micro-Hyperspec Sensor; Fluor Cam 800 MF	Simko *et al*., 2016 [55]
Magnetic Resonance Imaging	Tomato	Growing of truss	Homemade built	Windt *et al*., 2009 [65]
	Tomato	Maturity	1 T MR system	Zhang and McCarthy, 2012
	Cucumber	Internal freeze damage	9.4 T MR system	Kotwaliwale *et al*., 2012 [30]
	Bean	Pod water content	Homemade built	Rascher *et al*., 2011[51]
Pulse Amplitude Modulation fluorimetry	Bean	Photosyntetic traits, morphological parameters and shoot architecture	Growscreen Fluoro	Rascher *et al*., 2011 [51]
	Chinese cabbage	Quality	FluorPenFP 100 fluorimeter	Ptushenko *et al*., 2011
	Melon	Grafting compatibility	Imaging-PAM fluorometer	Calatayud *et al*., 2013 [9]
3D imaging	Tomato	Canopy	Digital camera	Aguilar *et al*., 2008 [1]
Positron Emission Tomography	Tomato	Carbon trans-location	microPET scanner	Kawachi *et al*., 2009 [29]

Imaging Platforms and Softwares

The success of phenomics depends upon their accurate results for which the data management and their analysis are of prime concern. There are few challenges for the data management in the phenomics research: firstly the data management services must have the ability to manage huge amount of the heterogeneous data in various formats (image, text and video), secondly, in order to facilitate the effective search, dissemination and query, the database management series within itself must have the ability to support the metadata related service to provide the structure and context of the data. In phenomics, for the meaningful evaluation of data and statistical analysis, a standard device for data storage is essential. For which the databases must be developed for each crop. The databases LycoTILL and Tomato mutant database are developed for tomato (Minoia *et al.*, 2010 and Menda *et al.*, 2004) [40, 38] and the database SGN was developed for solanaceae species (Bombarely *et al.*, 2011) [7]. The large data acquired from HTTPs must be robustly and accurately calibrated, reconstructed and then analyzed for which there is a requirement of specialized image understanding and the quantification algorithms. Several image analyses tools and software's (Table 2) have been developed for extracting different phenotypic traits.

Table 2: Different software's used in high throughput crop phenotyping (Rahman *et al.*, 2015) [49]

Tissue	Software	Parameters Measured	Reference
Roots	KineRoot	Measures curvature and root growth	Basu *et al.*, 2007 [6]
	PlaRoM	Measures growth traits and root extension under circadian or diurnal growth rhythms	Yazdanbakhsh and Fisahn, 2009 [73]
	EZ-Rhizo	2D analysis of root system architecture	Armengaud *et al.*, 2009 [3]
	Growscreen-Rhizo	Shoot biomass evaluation and root architecture parameters in 2D	Nagel *et al.*, 2012 [43]
	Roottrace	Measure of curvature and root length	Naeem *et al.*, 2011 [42]
	Dart	2D analysis of root system architecture	Le Bot *et al.*, 2010 [32]
	Smartroot	Architecture for complex root systems and Quantification of root growth	Lobet *et al.*, 2011 [35]
	Rootreader3d	3D analysis of root system architecture	Clark *et al.*, 2011 [12]
	Growth Explorer	2D analysis of root growth patterns	Basu and Pal, 2012 [5]
	Rootrak	3D root architecture of soil grown plant	Mairhofer *et al.*, 2012 [37]
Shoot/leaves	Traitmill	Platform to test the effect of plant-based transgenes on agronomically traits	Reuzeau *et al.*, 2006 [53]
	Phenopsis	Automated measurement of water deficit-related traits like leaf area, leaf number, transpiration rate and root growth	Granier *et al.*, 2006 [21]
	Leafanalyser	Analyzes of variation in leaf shape	Weight *et al.*, 2007
	Lamina	Measures leaf size and shape	Bylesjo *et al.*, 2008
	Hypotrace	Measures hypocotyls hook angle and growth rate	Wang *et al.*, 2009 [63]
	HTPheno	Measures the plant height, width and the projected shoot area	Hartmann *et al.*, 2011 [23]

	Leafprocessor	Measures different geometries of leaf	Backhaus *et al*., 2010 [4]
	LEAF-GUI	Analyzes macroscopic structure of leaves veins	Price *et al*., 2011 [47]
Seeds/grain	SHAPE	Extracts the contour shape from a full color bitmap image	Iwata *et al*., 2010 [26]
	ImageJ	General image analysis software for shape, area, and size; applied to grain	Herridge *et al*., 2011 [24]
	SmartGrain	High-throughput seed shape measurement	Tanabata *et al*., 2012 [60]

Conclusion

In the era of "omics" sciences several disciplines are integrated to solve different biological process, where modern plant phenotyping objective is to deliver high throughput and accurate results of the plant phenotype. In order to achieve this objective, sensing technologies, automation, databases and software's have been developed in recent years. The number of studies carried out in the vegetable crops have been rapidly increasing. Digital imaging techniques had paved the advancement and allowed the investigation of different aspects of crop including the yield, stress and pathological traits. It also aids in the assessment of nutrient status of the vegetable crop. As all the types of imaging techniques are been rapidly adapted for the plant phenotyping, the "big data" analytics in phenomics has become a serious issue and this problem needs an intensive research in near future. Besides the application of plant phenotyping in breeding, their continuous, fast and precise nature in protected cultivation of vegetable would help them in evaluation of novel precision management technique with a positive effect on environment and economic sustainability.

Reference

1. Aguilar MA, Pozo JL, Aguilar FJ, Sanchez-Hermosilla J, Páez FC, Negreiros J. 3D surface modelling of tomato plants using close-range photogrammetry. International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences. Beijing, 2008, 37.
2. Apan A, Datt B, Kelly R. Detection of pests and diseases in vegetable crops using hyperspectral sensing: a comparison of reflectance data for different sets of symptoms. In Proceedings of the 2005 Spatial Sciences Institute Biennial Conference 2005: Spatial Intelligence, Innovation and Praxis (SSC2005) Spatial Sciences Institute. 2005, 10-18.
3. Armengaud P, Zambaux K, Hills A, Sulpice R, Pattison RJ, Blatt MR *et al*. EZ-Rhizo: integrated software for the fast and accurate measurement of root system architecture. The Plant Journal, 2009; 57(5):945-956.
4. Backhaus A, Kuwabara A, Bauch M, Monk N, Sanguinetti G, Fleming A. LEAFPROCESSOR: a new leaf phenotyping tool using contour bending energy and shape cluster analysis. New phytologist, 2010; 187(1):251-261.
5. Basu P, Pal A. A new tool for analysis of root growth in the spatio-temporal continuum. New Phytologist, 2012; 195(1):264-274.
6. Basu P, Pal A, Lynch JP, Brown KM. A novel image-analysis technique for kinematic study of growth and curvature. Plant physiology, 2007; 145(2):305-316.
7. Bombarely A, Menda N, Tecle IY, Buels RM, Strickler S, Fischer-York T, *et al*. The Sol Genomics Network (solgenomics. net): growing tomatoes using Perl. Nucleic acids research, 2011; 39(1)D1149-D1155.
8. Bylesjö 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(1):82.
9. Calatayud Á, San Bautista A, Pascual B, Maroto JV, López-Galarza S. Use of chlorophyll fluorescence imaging as diagnostic technique to predict compatibility in melon graft. Scientia horticulturae, 2013; 149:13-18.
10. Chaerle L, Hagenbeek D, Vanrobaeys X, Van Der Straeten D. Early detection of nutrient and biotic stress in *Phaseolus vulgaris*. International Journal of Remote Sensing, 2007; 28(16):3479-3492.
11. Chiu YC, Hsu WC, Chang YC. Detecting cabbage seedling diseases by using chlorophyll fluorescence. Engineering in agriculture, environment and food, 2015; 8(2):95-100.
12. 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.
13. Clément A, Dorais M, Vernon M. Multivariate approach to the measurement of tomato maturity and gustatory attributes and their rapid assessment by Vis− NIR spectroscopy. Journal of agricultural and food chemistry, 2008; 56(5):1538-1544.
14. Cook JP, McMullen MD, Holland JB, Tian F, Bradbury P, Ross-Ibarra J *et al*, Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels. Plant physiology. 2012; 158(2):824-834.
15. Corti M, Gallina PM, Cavalli D, Cabassi G. Hyperspectral imaging of spinach canopy under combined water and nitrogen stress to estimate biomass, water, and nitrogen content. Biosystems engineering, 2017; 158:38-50.
16. Devacht S, Lootens P, Baert J, Van Waes J, Van Bockstaele E, Roldán-Ruiz I. Evaluation of cold stress of young industrial chicory (*Cichorium intybus* L.) plants by chlorophyll a fluorescence imaging. I. Light induction curve. Photosynthetica, 2011; 49(2):161-171.
17. Diezma B, Lleó L, Roger JM, Herrero-Langreo A, Lunadei L, Ruiz-Altisent M. Examination of the quality of spinach leaves using hyperspectral imaging. Postharvest biology and technology, 2013; 85:8-17.
18. Ecarnot M, Bączyk P, Tessarotto L, Chervin C. Rapid phenotyping of the tomato fruit model, Micro-Tom, with a portable VIS–NIR spectrometer. Plant physiology and biochemistry, 2013; 70:159-163.
19. Furbank RT, Tester M. Phenomics–technologies to relieve the phenotyping bottleneck. Trends in plant science, 2011; 16(12):635-644.
20. Gorbe E, Calatayud A. Applications of chlorophyll fluorescence imaging technique in horticultural research: a review. Scientia Horticulturae, 2012; 138:24-35.
21. 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(3):623-635.
22. Hahn F. Spectral bandwidth effect on a *Rhizopus stolonifer* spores detector and its on-line behavior using

red tomato fruits. Canadian Biosystems Engineering, 2004; 46:49-54.
23. Hartmann A, Czauderna T, Hoffmann R, Stein N, Schreiber F. HTPheno: an image analysis pipeline for high-throughput plant phenotyping. BMC bioinformatics, 2011; 12(1):148.
24. Herridge RP, Day RC, Baldwin S, Macknight RC. Rapid analysis of seed size in Arabidopsis for mutant and QTL discovery. Plant methods, 2011; 7(1):3.
25. Houle D, Govindaraju DR, Omholt S. Phenomics: the next challenge Nature Reviews Genetics 2010, 11.
26. Iwata H, Ebana K, Uga Y, Hayashi T, Jannink JL. Genome-wide association study of grain shape variation among *Oryza sativa* L. germplasms based on elliptic Fourier analysis. Molecular Breeding, 2010; 25(2):203-215.
27. Jackson SA, Iwata A, Lee SH, Schmutz J, Shoemaker R. Sequencing crop genomes: approaches and applications. New Phytologist, 2011; 191(4):915-925
28. Jahnke S, Menzel MI, Van Dusschoten D, Roeb GW, Bühler J, Minwuyelet S *et al*. Combined MRI–PET dissects dynamic changes in plant structures and functions. The Plant Journal, 2009; 59(4):634-644.
29. Kawachi N, Kikuchi K, Suzui N, Ishii S, Fujimaki S, Ishioka NS, Watanbe H. June. Imaging for carbon translocation to a fruit of tomato with carbon-11-labeled carbon dioxide and positron emission tomography. In 2009 1st International Conference on Advancements in Nuclear Instrumentation, Measurement Methods and their Applications IEEE. 2009, 1-5.
30. Kotwaliwale N, Curtis E, Othman S, Naganathan GK, Subbiah J. Magnetic resonance imaging and relaxometry to visualize internal freeze damage to pickling cucumber. Postharvest biology and technology, 2012; 68:22-31.
31. Kumar J, Pratap A, Kumar S. eds., Phenomics in crop plants: Trends, options and limitations New Delhi: Springer India, 2015; 8:296.
32. Le Bot J, Serra V, Fabre J, Draye X, Adamowicz S, Pagès L. DART: a software to analyse root system architecture and development from captured images. Plant and Soil, 2010; 326(1, 2):261-273.
33. Lee WS, Alchanatis V, Yang C, Hirafuji M, Moshou D. Li C. Sensing technologies for precision specialty crop production. Computers and electronics in agriculture, 2010; 74(1):2-33
34. Li L, Zhang Q, Huang D. A review of imaging techniques for plant phenotyping. Sensors, 2014; 14(11):20078-20111.
35. Lobet G, Pagès L, Draye X. A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant physiology, 2011; 157(1):29-39.
36. Lootens P, Devacht S, Baert J, Van Waes J, Van Bockstaele E, Roldàn-Ruiz I. Evaluation of cold stress of young industrial chicory (*Cichorium intybus* L.) by chlorophyll a fluorescence imaging. II. Dark relaxation kinetics. Photosynthetica, 2011; 49(2):185-194.
37. Mairhofer S, Zappala S, Tracy SR, Sturrock C, Bennett M, Mooney SJ *et al*. RooTrak: automated recovery of three-dimensional plant root architecture in soil from X-ray microcomputed tomography images using visual tracking. Plant physiology, 2012; 158(2):561-569.
38. Menda N, Semel Y, Peled D, Eshed Y, Zamir D. *In silico* screening of a saturated mutation library of tomato. The Plant Journal, 2004; 38(5):861-872.
39. Meroni M, Rossini M, Guanter L, Alonso L, Rascher U, Colombo R *et al*. Remote sensing of solar-induced chlorophyll fluorescence: Review of methods and applications. Remote Sensing of Environment. 2009; 113(10):2037-2051.
40. Minoia S, Petrozza A, D'Onofrio O, Piron F, Mosca G, Sozio G *et al*. A new mutant genetic resource for tomato crop improvement by TILLING technology. BMC research notes, 2010; 3(1):69.
41. Mishra Y, Jänkänpää HJ, Kiss AZ, Funk C, Schröder WP, Jansson S. Arabidopsis plants grown in the field and climate chambers significantly differ in leaf morphology and photosystem components. BMC Plant Biology, 2012; 12(1):6.
42. Naeem A, French AP, Wells DM, Pridmore TP. High-throughput feature counting and measurement of roots. Bioinformatics. 2011; 27(9):1337-1338.
43. Nagel KA, Putz A, Gilmer F, Heinz K, Fischbach A, Pfeifer J, *et al*. GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons. Functional Plant Biology. 2012; 39(11):891-904.
44. Pierret A, Capowiez Y, Belzunces L, Moran CJ. 3D reconstruction and quantification of macropores using X-ray computed tomography and image analysis. Geoderma, 2002; 106(3, 4):247-271.
45. Pineda M, Pérez-Bueno ML, Barón M. Detection of bacterial infection in melon plants by classification methods based on imaging data. Frontiers in plant science. 2018; 9:164.
46. Polder G, Van der Heijden GWAM, Young IT. Hyperspectral image analysis for measuring ripeness of tomatoes. In Proceedings of the ASAE Annual International Meeting, Milwaukee, WI, USA, 2000.
47. Price CA, Symonova O, Mileyko Y, Hilley T, Weitz JS. Leaf extraction and analysis framework graphical user interface: segmenting and analyzing the structure of leaf veins and areoles. Plant Physiology. 2011; 155(1):236-245.
48. Ptushenko VV, Avercheva OV, Bassarskaya EM, Berkovich YA, Erokhin AN, Smolyanina SO *et al*. Possible reasons of a decline in growth of Chinese cabbage under a combined narrowband red and blue light in comparison with illumination by high-pressure sodium lamp. Scientia Horticulturae. 2015; 194:267-277.
49. Rahman H, Ramanathan V, Jagadeeshselvam N, Ramasamy S, Rajendran S, Ramachandran M *et al*. Phenomics: technologies and applications in plant and agriculture. In Plant Omics: The Omics of Plant Science Springer, New Delhi. 2015, 385-411.
50. Rascher U, Pieruschka R. Spatio-temporal variations of photosynthesis: the potential of optical remote sensing to better understand and scale light use efficiency and stresses of plant ecosystems. Precision Agriculture. 2008; 9(6):355-366.
51. Rascher U, Blossfeld S, Fiorani F, Jahnke S, Jansen M, Kuhn AJ *et al*. Non-invasive approaches for phenotyping of enhanced performance traits in bean. Functional Plant Biology. 2011; 38(12):968-983.
52. Ray DK, Mueller ND, West PC, Foley JA. Yield trends are insufficient to double global crop production by 2050. PloS one. 2013; 8(6):e66428
53. Reuzeau C, Frankard V, Hatzfeld Y, Sanz A, Van Camp

W, Lejeune P *et al*. Traitmill™: a functional genomics platform for the phenotypic analysis of cereals. Plant Genetic Resources. 2006; 4(1):20-24.
54. Rodríguez-Moreno L, Pineda M, Soukupová J, Macho AP, Beuzón CR, Barón M *et al*. Early detection of bean infection by *Pseudomonas syringae* in asymptomatic leaf areas using chlorophyll fluorescence imaging. Photosynthesis research. 2008; 96(1):27-35.
55. Simko I, Hayes RJ, Furbank RT. Non-destructive phenotyping of lettuce plants in early stages of development with optical sensors. Frontiers in plant science. 2016; 7:1985.
56. Sirault XR, James RA, Furbank RT. A new screening method for osmotic component of salinity tolerance in cereals using infrared thermography. Functional Plant Biology. 2009; 36(11):970-977.
57. Stuppy WH, Maisano JA, Colbert MW, Rudall PJ, Rowe TB. Three-dimensional analysis of plant structure using high-resolution X-ray computed tomography. Trends in plant science. 2003; 8(1):2-6.
58. Szuvandzsiev P, Helyes L, Lugasi A, Szántó C, Baranowski P, Pék Z. Estimation of antioxidant components of tomato using VIS-NIR reflectance data by handheld portable spectrometer. International Agrophysics, 2014, 28(4).
59. Tamburini E, Costa S, Rugiero I, Pedrini P, Marchetti MG. Quantification of lycopene, β-carotene, and Total soluble solids in intact red-flesh watermelon (*Citrullus lanatus*) using on-line Near-Infrared Spectroscopy. Sensors. 2017; 17(4):746.
60. Tanabata T, Shibaya T, Hori K, Ebana K, Yano M. SmartGrain: high-throughput phenotyping software for measuring seed shape through image analysis. Plant physiology, 2012; 160(4):1871-1880.
61. Tripodi P, Massa D, Venezia A, Cardi T. Sensing technologies for precision phenotyping in vegetable crops: current status and future challenges. Agronomy, 2018; 8(4):57.
62. Van As H, Van Duynhoven J. MRI of plants and foods. Journal of Magnetic Resonance. 2013; 229:25-34.
63. Wang L, Uilecan IV, Assadi AH, Kozmik CA, Spalding EP. HYPOTrace: image analysis software for measuring hypocotyl growth and shape demonstrated on Arabidopsis seedlings undergoing photomorphogenesis. Plant physiology, 2009; 149(4):1632-1637.
64. Weight C, Parnham D, Waites R. Technical Advance: LeafAnalyser: a computational method for rapid and large-scale analyses of leaf shape variation. The Plant Journal. 2008; 53(3):578-586.
65. Windt CW, Gerkema E, Van As H. Most water in the tomato truss is imported through the xylem, not the phloem: a nuclear magnetic resonance flow imaging study. Plant physiology. 2009; 151(2):830-842.
66. Xie L, Ying Y, Ying T. Quantification of chlorophyll content and classification of nontransgenic and transgenic tomato leaves using visible/near-infrared diffuse reflectance spectroscopy. J Agric. Food Chem. 2007; 55:4645-4650.
67. Xing J, Ngadi M, Wang N, De Baerdemaeker J. Wavelength selection for surface defects detection on tomatoes by means of a hyperspectral imaging system. In 2006 ASAE Annual Meeting American Society of Agricultural and Biological Engineers. 2006, 1.
68. Xu HR, Ying YB, Fu XP, Zhu SP. Near-infrared spectroscopy in detecting leaf miner damage on tomato leaf. Biosystems Engineering. 2007; 96(4):447-454.
69. Xu HR, Yu P, Fu XP, Ying YB. On-site variety discrimination of tomato plant using visible-near infrared reflectance spectroscopy. Journal of Zhejiang University Science B, 2009; 10(2):126-132.
70. Xue L, Yang L. Deriving leaf chlorophyll content of green-leafy vegetables from hyperspectral reflectance. ISPRS Journal of Photogrammetry and Remote Sensing, 2009; 64(1):97-106.
71. Yang HQ. Nondestructive prediction of optimal harvest time of cherry tomatoes using VIS-NIR spectroscopy and PLSR calibration. In Advanced Engineering Forum Trans Tech Publications Ltd. 2011; 1:92-96.
72. Yang HY, Inagaki T, Ma T, Tsuchikawa S. High-resolution and non-destructive evaluation of the spatial distribution of nitrate and its dynamics in spinach (*Spinacia oleracea* L.) Leaves by near-infrared hyperspectral imaging. Frontiers in plant science. 2017; 8:1937.
73. Yazdanbakhsh N, Fisahn J. High throughput phenotyping of root growth dynamics, lateral root formation, root architecture and root hair development enabled by PlaRoM. Functional Plant Biology. 2009; 36(11):938-946.
74. Zhang L, McCarthy MJ. Measurement and evaluation of tomato maturity using magnetic resonance imaging. Postharvest Biology and Technology. 2012; 67:37-43.
75. Zhang Y, Zhang N. Imaging technologies for plant high-throughput phenotyping: A review. Front. Agric. Sci. Eng. 2018; 5:406-419
76. Zhang Y, Zhao C, Du J, Guo X, Wen W, Gu S *et al*. Crop phenomics: current status and perspectives. Frontiers in Plant Science. 2019; 10:714.
77. Zhu S, Feng L, Zhang C, Bao Y, He Y. Identifying Freshness of Spinach Leaves Stored at Different Temperatures Using Hyperspectral Imaging. Foods. 2019; 8(9):356.