High-throughput crop phenotyping in vegetable crops

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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 technologies, 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) [78].

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.

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].

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:
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) [36, 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) [44]. 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) [28]. 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 13C, 1H, 14N and 14N. 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 52Fe, 13N or 13C are non-invasively imaged. During photosynthesis when CO2 are consumed, a 3D image of 13C-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, 55].

| 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 2TM | Szuvandzisiev 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 [58] |
| | 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 dadiantii 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] |
In phenomics, for the meaningful evaluation of data and related service to provide the structure and context of the data. Within itself must have the ability to support the metadata dissemination and query, the database management system must have the ability to manage huge amount of the heterogeneous data in various formats (image, text and video), for which the data management and their analysis are of prime concern. There are few challenges for the data management in phenomics research: firstly the data management services are developed for tomato (Minoia et al., 2010 and Menda et al., 2004) 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.

### 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) [6] 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 |
|--------|----------|----------------------|-----------|
| Kineto | PlantViz  | Mesures curvature and root growth | Basu et al., 2007 [6] |
| Plants | PlaRoM   | Mesures growth traits and root extension under circadian or diurnal growth rhythms | Yazdanbakhsh and Fisahn, 2009 [7] |
| EZ-Rhizo | Growscreen-Rhizo | 2D analysis of root system architecture | Armengaud et al., 2009 [8] |
| Rootrace | Rootmeasure | Shoot biomass evaluation and root architecture parameters in 2D | Nagel et al., 2012 [9] |
| Smartroot | 2D analysis of root architecture | Le Bot et al., 2010 [10] |
| Rootreader3d | 3D analysis of root system architecture | Lobet et al., 2011 [11] |
| Growth Explorer | 2D analysis of root growth patterns | Clark et al., 2011 [12] |
| RootRaker | 3D root architecture of soil grown plant | Basu and Pal, 2012 [13] |
| Shoot/leaves | Traitmill | Platform to test the effect of plant-based transgenes on agronomically traits | Reuzeau et al., 2006 [14] |
| Phenopsis | Automated measurement of water deficit-related traits like leaf area, leaf number, transpiration rate and root growth | Granier et al., 2006 [15] |
| Leafanalyzer | Analyzes of variation in leaf shape | Weight et al., 2007 |
| Lamina | Measures leaf size and shape | Bylesio et al., 2008 |
| Hypotrace | Measures hypocotyls hook angle and growth rate | Wang et al., 2009 [16] |
| HTPPheno | Measures the plant height, width and the projected shoot area | Hartmann et al., 2011 [17] |
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 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.

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