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Phenomics: Approaches and Application in Crop Improvement

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Phenotype is the combination of genotype and environment where the plant grows. Phenomics is a way of speeding up phenotyping with the help of high-tech imaging systems and computing power. It has been a practice in plant breeding for selecting the best genotype after studying phenotypic expression in different environmental conditions and also using them in hybridization programs, to develop new improved genotypes. Phenomics share the advantages of faster evaluation, facilitating a more dynamic whole-of-lifecycle measurement with improved precision, being less dependent on periodic destructive assays and with reduced need for replication in the field. Phenomics aids to obtain high-dimensional phenotypic data on an organism at large scale with the various tools involved. Phenomics, however is more than just data collection paired with data mining. It is a comprehensive approach that combines systems biology and statistical correlation. Data mining techniques and big data approaches have the power to create knowledge that cannot be created otherwise with accurate precision manually.

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1. INTRODUCTION

Plant phenotyping has been a part of crop and variety selection since the time of human civilization when humans selected the best individuals of a crop species for domestication [1]. A plant's genotype is the genetic constitution of an organisms and a plant's phenotype is how it looks and performs. Plant's phenotype is a combination of its genotype and the environment it grows in (Vp = Vg + Ve). Plants with the same genotype can have different phenotypes depending on the environmental conditions they are grown in [2-4]. It has been a practice in plant breeding for selecting the best genotype after studying phenotypic expression in different environmental conditions and also using them in hybridization programs, to develop new improved genotypes [5]. Phenomics share the advantages of faster evaluation, facilitating a more dynamic whole-of-lifecycle measurement with improved precision, being less dependent on periodic destructive assays and with reduced need for replication in the field. Phenomics aids to obtain high-dimensional phenotypic data on an organism at large scale.

Johannsen coined the terms 'genotype' and 'phenotype'. He demonstrated, variation in quantitative traits in genetically-identical material, thus proved that variation in a given observed traits is not controlled entirely by genetics. It led to origin of the word – "pheno". After 1950, 'phenotyping' as a noun, 'to phenotype' as a verb and 'phenome' as the collective noun were introduced, which have been accepted scientifically and are being utilized commonly in literature. The word 'phenome' refers to the phenotype as whole [6] i.e., expression of genome for a trait in a given environment. Ecologists used phenotyping to study phenotypic plasticity of genotypes and suggested the role of the genotype and environmental conditions in the expression of plant phenotypes under which it develops [7]. While phenomics refers to the high-dimensional phenotypic data on an organism at large scale is obtained, phenomics is used as an analogy to genomics. In genomics, complete characterization of a genome is possible while in phenomics, complete characterization of phenome is difficult due to the change in the phenotypic expression of traits over the environmental conditions [8].

2. IMPORTANCE OF PLANT PHENOMICS

By 2050, it is estimated that 9.1 billion people will populate the planet earth. And the need of high yielding varieties would give sustainable yields even under adverse and changing environmental conditions. Therefore, phenomics can act as a powerful tool to overcome one of humanity's greatest challenges: Hunger.

Biofuels are fuels such as ethanol and biodiesel that are produced from plant matter, and are called 'feedstocks'. Such crops could not only be eaten by people instead can be turned into fuel. These crops could compete with food crops for the best agricultural land. Hence, researchers are in need to make trials for alternative plant species as feedstock that can grow on less productive lands. But, then these crops will need to tolerate a wide range of environmental stresses, such as low water availability, salinity or low nutrient supplies, to be able to grow successfully in such 'marginal' lands. It was estimated that half of the increase in yield of the main agricultural crops has come through plant breeding processes [9,10].

Phenotyping can be a laborious process, taking many days, weeks or even months. The high-tech automated plant analysis systems of plant phenomics have speeded up traditional, time consuming methods of plant analysis. Phenomics is focused on coming up with practical solutions to problems that will affect us and our future. This technology can be used for study plants from the small scale to studying individual cells or leaves to up to the large scale of an entire ecosystem.

Phenomics technology allows plants, which are only one month old, move through a series of chambers, each one automatically taking different images and measurements. Within a few hours, the high-tech imaging and computing systems automatically measure the plants and the necessary data are given. The young plants with the characteristics the researcher looking for can be selected and grown on to mature for seed; thereby, the rest can be discarded.

The scientific accuracy of molecular breeding is strongly related to phenomics and therefore phenotyping [11]. The main aim of phenomics is to connect between genetic, plant function, and agricultural characteristics [12]. Performing of sensor technologies and algorithmic applications for automatic phenotyping are being handled to overcome the defect of the manual techniques [13]. Agro-morphological traits should be universal. If not, different and inconsistent characterization might lead to misunderstanding for breeding studies. Hence, it states the importance of phenotyping for the identification and utilisation of better cultivars.

The science of genomics which is the study of genes has produced masses of information. But, a ‘bottleneck’ has developed in which developments based on genomic information are unable to keep pace with the huge amount of data that genomics produces daily. Phenomics can translate this information into useful applications. Phenomics links the resulting data to *gene* sequence information. In this way, gene discovery can be put to good use by the faster development of useful crop varieties. The plant phenomic developments are actually helping to make simply plant physiology in ‘new clothes’.

Phenotyping is generally a technically challenging, requires destructive harvesting at fixed time, slow and costly. Phenotyping tools and techniques are non-invasive like, spectroscopy, image analysis, robotics and high-performance computing for phenotyping. Phenomics researchers are not just include biologists, chemists, physicists, computer scientists, engineers, mathematicians, physiologists, microscopists, geneticists, and plant breeders work but also all these sciences come together to develop new phenomics based phenotyping methods.

Hence, this science is one of the best in its kind and shares the following objectives:

1. It can be used in laboratories and also in fields to analyse phenotypes in natural conditions as well as under controlled environment
2. Evaluation can be faster, and facilitates a more dynamic whole-of-lifecycle measurement
3. Less dependent on periodic destructive assays
4. Improved the precision in recording the data
5. Reduce the need for replication in the field

2.1 Types of Phenomics

2.1.1 Forward phenomics

Forward phenomics is a way of finding the plant varieties that are the ‘best of the best’. It speeds up plant breeding by screening large number of plants at the seedling stage using automated imaging technology. This makes it faster to identify interesting traits, as the plants do not have to be grown to an adult stage in the field.

2.1.2 Reverse phenomics

Reverse phenomics is pulling the ‘best’ varieties apart to discover why they are the best. The phenotype or desired trait such as “drought tolerance” is already known. Researchers then try to work out the mechanisms that control the trait and the gene or genes that are responsible for it.

Plant phenotyping technology involves two insights, *i.e.,* looking inward and looking outward. Looking inward involves the study of leaves and roots with hi-tech imaging systems and Looking outward involves the study of plant growth in controlled conditions and in the field. It gives an overall performance of plants.

2.2 Plant Phenomics Tools

Phenomics borrows imaging techniques from medicine to allow researchers to study the inner workings of leaves, roots or whole plants. It includes the following tools;

2.2.1 Three-dimensional (3D) imaging

Digital photos of the top and sides of plants are combined into a 3D image. Measurements that can be taken using a 3D image include: shoot mass, leaf number, shape and angle, leaf colour and leaf health. Technically, pots of plants move on a conveyor belt through an imaging chamber and 3D models are automatically generated by a computer program. Obtained images are transferred to the software and required editions as colour improving and optimization are made. Digital images have advantages such as simple recording, transmitting, and storing in a database. However, algorithms are necessary to gather and analyze the huge amount of data [14].

The TrayScan system holds, tray labelled with a barcode so that plants can be easily identified and 3D images are taken appropriately. Early detection of diseased plants with modern vision techniques can significantly reduce costs. The virus often infects many tissues, if not the whole plant. In contrast, bacteria affects the vascular bundles especially in lower parts of the stem, causing symptoms as black-rot in the stem, and general symptoms like yellowing, necrosis and wilting of the leaves on the affected stems.

In 2016 a new experiment was set up, using 49 bacterial diseased potato plants and 20 control plans grown in the field. During the growing season, each week hyperspectral imaging and a full 3D scan was made, as well as a top view RGB-depth scan was done. Preliminary results show that plants affected by bacterial diseases are distinguishable from healthy plants using a combination of the three data modalities [15].

2.2.2 Far-infrared (FIR) imaging

This system uses light in the FIR region of the spectrum (15 µm to 1 mm) to study the temperature. Temperature differences can be used to study the salinity tolerance, water usage, photosynthesis efficiency *etc.* Cooler plants have better root systems and take up more water. Far-infrared light can be shone on plants growing in high-tech growth cabinets to create ‘heat maps’ of each leaf. These heat maps help to study the temperature differences.

2.2.3 Near infra red (NIR) imaging

The NIR region of the spectrum is used to measure water content and its movement in leaves and soil.

Shortwave infrared hyperspectral (950–1650 nm) imaging system was explored to detect sour skin (*Burkholderia cepacia*) a major postharvest disease in onions on both the healthy and infected onions. Principal component analysis (PCA) revealed that neck area of the onion at two wavelengths (1070 and 1400 nm) was most indicative of the sour skin. Using the pixel number of the segregated areas, Fisher’s discriminant analysis recognized 80% healthy and sour skin-infected onions were recognised. The result of this study can be used to further develop a multispectral imaging system to detect sour skin-infected onions on packing lines [16].

A pixel-wise mapping of spectral reflectance in the visible and near-infrared range enabled the detection and detailed description of diseased tissue on the leaf level. Leaf structure was linked to leaf spectral reflectance patterns. Depending on the interaction with the host tissue, the pathogens caused disease-specific spectral signatures. Due to a pixel-wise extraction of pure spectral signatures a better understanding of changes in leaf reflectance caused by plant diseases was achieved using hyperspectral imaging for analysis of symptoms caused by different sugar beet diseases *viz., Cercospora* leaf spot, powdery mildew and leaf rust at different development stages [17].

2.2.4 Fluorescence imaging

Fluorescence occurs when an object absorbs light of one wavelength and gives off light of a different wavelength. A computer program converts the resulting fluorescence into false-colour signals to allow instant analysis of plant health. Chlorophyll fluorescence is used to study the effect of different genes or environmental conditions on the efficiency of photosynthesis and stress monitoring.

Fig. 1. Photographs of crop cultivation in laboratory

The measurement of chlorophyll fluorescence of greenhouse-grown cucumber (*Cucumis sativus* L. cv. Mustang) fruit can be a useful tool in monitoring senescence, temperature stress, and desiccation during storage [18]. Pre-symptomatic monitoring clearly opens perspectives for quantitative screening for disease resistance. These non-destructive imaging techniques were able to visualize infections at an early stage before damage appeared. Under growth-room conditions, a robotized set-up captured time series of visual, thermal, and chlorophyll fluorescence images from infected regions on attached leaves [19].

2.2.5 Magnetic resonance imaging (MRI)

The MRI uses a magnetic field and radio waves to take images of roots in the same way as same way as it takes images of organs and soft tissues in medical applications. MRI allows the 3D geometry of roots to be viewed just as if the plant is growing in the soil and also describes 3D representation of water movement.

In situ magnetic resonance imaging of plant roots was studied to check the different developmental stages of *Pisum sativum* (pea) seed germination. The *Pisum sativum* leaf metabolism was profiled, using 1D and 2D nuclear magnetic resonance (NMR) spectroscopy to monitor the changes induced by drought-stress under both glasshouse and simulated field conditions. Significant changes in resonances were attributed to a range of compounds, identified as both primary and secondary metabolites, highlighting metabolic pathways that are stress-responsive. The metabolites present at higher concentrations in drought-stressed plants under all growth conditions included proline, valine, threonine, homoserine, myoinositol, γ-aminobutyrate (GABA) and trigonelline (nicotinic acid betaine). Such changes may be expected to impact both on plant performance and crop end-use [20].

2.2.6 Spectral reflectance

Spectral reflectance is the fraction of light reflected by a non-transparent surface. Depending on the part of the electromagnetic spectrum that is used to analyse reflectance, different matter can have different patterns of reflectance. This is called the spectral signature. It is used to determine the chemical composition of plants such as: levels of chlorophyll and other pigments in leaves, water-soluble carbohydrates and nitrogen in leaves, stems, and the chemical composition of plants *etc*. A video camera is used for plant detection. An on-the-screen-display generates a video sequence and includes the measured data. As a consequence, the data can be interpreted together with an image and a clear correlation.

Phenomics researchers can use spectral reflectance technology to monitor several plant properties in the field at the same time. This means that researchers can determine the biochemical composition of crop plants without having to destroy the plants by harvesting. Researchers can use spectral reflectance to tell if a plant is stressed by saline soil or drought, well before it can be seen by eye.

Discrimination between potato tubers and clods by detecting the significant wavebands was done using the RGB colour camera. The discrimination was performed using the linear discriminant analysis based on the colour information in the pixels. Pixels of the wet objects were discriminated with a success rate of 92% and by 73% for dry objects. It was found that the success rate of discrimination was also based on varying rate of wavebands [21].

Deriving leaf chlorophyll content of green-leafy vegetables from hyperspectral reflectance using four different leafy vegetables of varying colours of different wavelengths was performed by [22].

Thus, like genomic platforms, phenotyping platforms have also developed databases such as the plant meta-phenomics database [23], the Plant Trait database TRY (http://www.try-db.org, accessed September 2012) etc., they bring together phenotypic responses to the environment for a wide range of plant traits and parameters. These phenotyping database along with available international genomic databases (TAIR, TIGR and NCBI, and with other 'omics' information such as metabolomic, proteomic and transcriptomic data) are an important tools to understand the genetic architecture of complex traits.

2.3 Phenomics in Field

The hi-tech computer based imaging systems cannot only be used in controlled conditions or in a limited area, but also be used in field's at large scale. The phenomic remote sensing technology allows researchers to study plants in the field. Measurements can be taken on many plants at once and over a whole growing season.

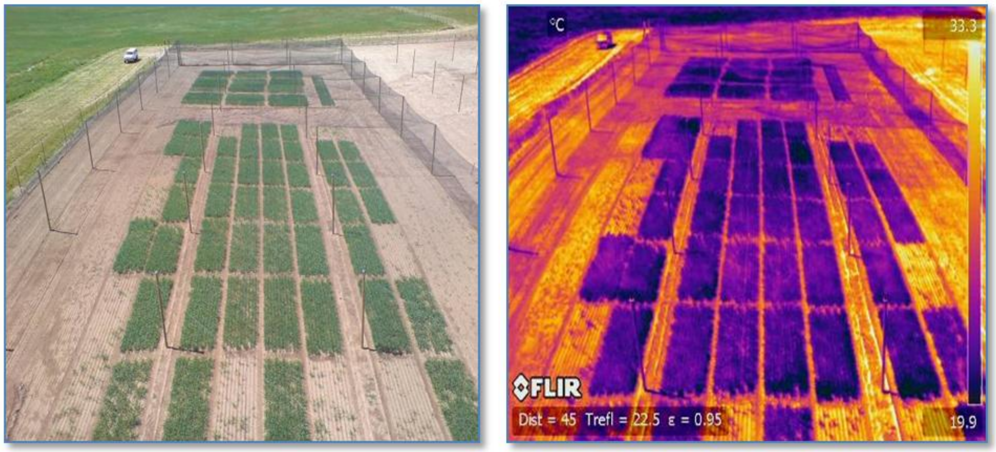

Fig. 2. Hi-tech computer based imaging of plants in the field

Phenonet sensor network, phenomobile, phenotower, are some of the important tools which allow plant phenotyping in the field to study large number of plants, simultaneously.

2.3.1 Phenonet sensor network

A network of data loggers collects information from the field of crops and sends it through the mobile phone network to researchers at the lab. It saves daily visits to field sites, which is especially useful if the site is in a remote area. Sensors include; far infrared thermometer, weather sensor, soil moisture sensor, thermistor (soil temperature). The phenonet's modules are self-contained PVC tubes containing a battery, sensors, radio transmitter and microprocessor. They take measurements every 10 seconds, and average the measurements over 5-minute.

2.3.2 Phenomobile

The phenomobile is a modified golf buggy that moves through a field of plants, taking measurements as it travels along. It can travel 3–5 km per hour. Phenomobile, reveals the temperature of the leaves and also the other characteristics using the sensors.

The system is currently providing a throughput around 150 micro-plots per hour, allowing sampling around 1000 micro-plots within a day. The system may also run during the night while variables are significantly reducing the data volume. The phenomobile carries equipment to measure: digital cameras, far infrared cameras, stereo-imaging system of two digital cameras with three lasers to create 3D reconstructions of plots - measure leaf area, the volume (biomass) of plants, height and plant density and a laser that shines red light onto the plants in combination with spectral reflectance - crop's chemical composition.

2.3.3 Phenotower

The phenotower is used to take images of crops 16 m above the ground level.The phenotower allows researchers to take images of many plants at once. The data is used to compare canopy temperature, leaf greenness and groundcover between different plant lines at the same time.

2.3.4 Multicopter

The multicopter can take images of a field from a few centimetres above the ground to a height of up to 100 metres. Multicopter will be equipped with a computer, a GPS, and colour and infrared cameras. The infrared and colour images can be used to identify the relative differences in canopy temperature. It indicates plant water use most efficiently. The Multicopter is currently in a testing phase with a normal camera, with plans for further development.

2.3.5 Rhizotron

Root observation using rhizotrons are similar to the observation of roots in soil-filled pot, except that clear acrylic glass panels allow visual monitoring of root growth at the surface of the glass [24-26]. Variation in root growth and

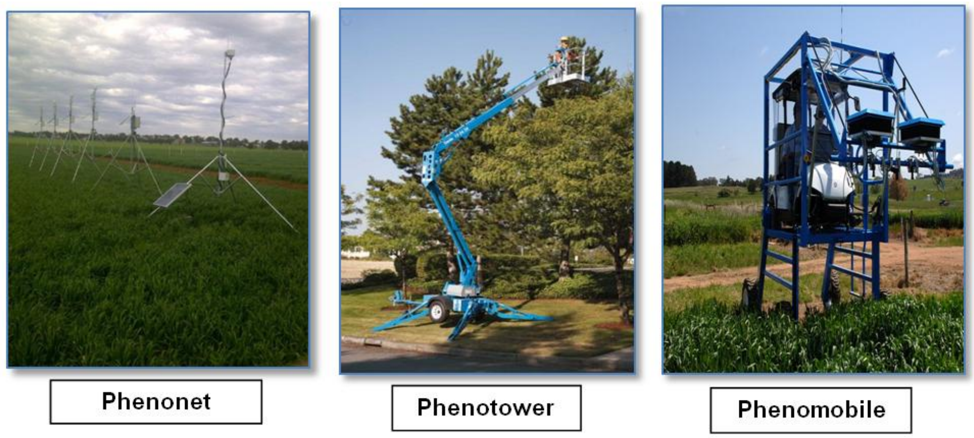

Fig. 3. Instrumental design

morphology among the tested crop plants can be traced on the outside surface of the acrylic glass using a marker pen, different colours may be used to indicate the presence of roots at successive time intervals, followed by scanning for root quantification.

Rhizotrons can be designed and constructed to meet specific research needs, such as for deep rooted crops and long growth periods having plants. Sampling of the root exudates around individual root tips is done using the anion exchange membrane (AEM). To analyse the root growth and architecture, researchers float the plants in water on a clear plastic tray, and use a flatbed scanner to take high-resolution, high-contrast images. A computer program transforms the images into knowledge by calculating root length and diameter, and analysing root branching patterns.

Various institutions throughout the world and also in India are working for phenomics. A list of few institutes working upon phenomics is mentioned in Table 1 and the programmes that were conducted in India are mentioned in Table 2.

Artificial vision systems are powerful tools for the automatic inspection of fruits and vegetables. Typical target applications of such systems include grading, quality estimation from external parameters or internal features, monitoring of fruit processes during storage or evaluation of experimental treatments.

The capabilities of an artificial vision system go beyond the limited human capacity to evaluate long-term processes objectively or to appreciate events that take place outside the visible electromagnetic spectrum and make it possible

Table 1. Phenomics programmes being carried all over the world

S. no.	Institute	Description
1.	IBERS	Located at Aberystwyth University
2.	International Plant Phenomics Network	A space for news about international phenomics activities
3.	Lepse,	Laboratory of plant ecophysiological responses to environmental stress located at Montpellier
4.	National Plant Phenomics Centre	Based at IBERS, only one in its kind in UK and only one in a few in the world to enable non-destructive imaging being practice
5.	Tools and Resources from- CPIB (Center for Plant Integrative Biology)	University of Nottingham
6.	Purdue University Institute for plant sciences	Located at USA
7.	The Australian Plant Facility	The high resolution plant phenomics center at Canberra

Table 2. Phenomics programmes in India

S. no.	Description
1.	3rd International Plant Phenotyping Symposium was held in Chennai, India on February 2014
2.	Inauguration of Plant Phenomics Facility at Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, was done on 1st July 2014. This unique state of the art facility has been installed under National Initiative on Climate Resilient Agriculture (NICRA) project launched by ICAR to develop adaptation and mitigation strategies to deal with climate change impacts on Indian agriculture.
3.	ICAR Sponsored Short Course on Non-destructive Phenotyping and Phenomics for Dissection of Abiotic Stress Tolerance, Gene Discovery and Crop Improvement (14-23 July, 2014) was organized by Department of Plant Physiology Indian Agricultural Research Institute.
4.	Inauguration of Plant Phenomics National Facility at ICAR in Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru was done on 1st November 2015
5.	Short Term Training Course on “Phenomics: Perspectives for Application in Improvement of Abiotic Stress Tolerance in Crop Plants” was held on July 20-29, 2017 NIASM at Maharashtra.

to explore defects or features that the human eye is unable to see.

Much of the data generated in high-throughput phenotyping platform are just mathematical transformations of numbers, and hence, it is difficult to understand those [27]. We do not even have a physical concept of what some of the numbers obtained by high-throughput phenotyping platforms mean in terms of plant or crop performance [28].

More user-friendly post-processing of the raw data generated is needed. Improved software tools to optimize automation and speed up robust data analysis should support such a trend [29].

3. CONCLUSION

Phenomics can be determined as “A Novel Big Data Approach”. Data mining techniques and big data approaches have the power to create knowledge that cannot be created otherwise. Only recently have people become aware of the impact these techniques and approaches. Advanced image analysis in digital form is a way to meaningfully structure images and extract statistical data from relevant objects, regions, and textures for subsequent data mining procedures. Phenomics extracts these data in a much more extensive manner than what can be done manually; enabling increased scientific insight and ultimately improving the plant breeding procedures. In particular the information is highly relevant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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