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Review

Plant phenomics: High-throughput technology for accelerating genomics

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Plant phenomics is a high-throughput path-breaking area that meets all the requirements for the collection of accurate, rapid and multi-faceted phenotypic data. Plant phenomics is an approach to envisage complex traits that are appropriate for selection, and provides relevant information as to why particular genotype can stand out in particular environmental conditions. The technique of plant phenotyping can be operated in various dimensions, from the gene to the whole-plant level under a specific environment, and management practices. Through this review, we discuss the recent advances in plant phenomics, highlighting different field and confined high-throughput technologies for utilization in forward and reverse genetics. These plant phenomics technique are very relevant in stress identification, study physiological processes, rapid and efficient screening, dissection and confirmation for understanding the genetic basis of different traits, genes and aspects. High-throughput phenomics technologies are essential to avoid human error and to reduce time consumption while phenotyping large germplasm populations, or for confirmation of gene or trait functional analysis.

Keywords. Forward and reverse genetics; imaging techniques; phenotype; plant phenomics

1. Introduction

The commonly used traditional phenotyping tools are generally low-throughput and labor-intensive. On the other hand, the use of next-generation genotyping and phenotyping technologies is advantageous over conventional breeding programs as these are rapid, accurate and help in accelerating crop breeding programs. Increasing yields under the vertical intensification scenario will involve novel approaches at the molecular level and plant breeding will boost both productivity and resource use efficiency. Under varied climatic conditions, development of novel and superior cultivar is of foremost importance, requiring assessment of thousands of breeding lines and this can be achieved by rapid and precise phenotypic assessment. Recent developments have identified phenomics as a path-breaking area to meet all the requirements that can be acquired with the help of high-throughput phenotyping (high-dimensional phenotypic data). The use of high-throughput plant phenomics approaches may useful to predict the composite characters that are associated (forward phenomics), and in addition, it provides information as to why particular genotypes/germplasm/line are distinct for a definite climatic condition (reverse phonemics). A phenotype can be defined as the characteristics of an organism which is a result of interaction to the environment, genotype and crop management. Plant phenomics are included the collection of relevant phenotypic data at various levels of organization, which will in turn facilitate a more complete characterization of phenotypic space produced by a specific set of genome or genomes. Therefore, plant phenotyping can be used at various levels of dimension

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and resolution in different climatic conditions (from controlled to field conditions) and from gene to whole-plant level. However, every level focuses on a specific trait and the final goal is to attain an integrated knowledge from each level (bottom to top) to produce a cultivar with superior performance. Of this, the application of plant phenotyping methods as a part of breeding programs has developed into an important research tool that facilitates breeders to develop cultivars with higher adaptability under different environmental conditions. The highlighted different fields and confined high-throughput technologies available in India using trait identification, dissection, and confirmation for understanding the genetic basis of diverse traits/aspects possibly will be useful in breeding for climate change.

The historical records on precipitation, stream flow, and drought indices altogether show an increased aridity since 1950 over many land areas (Dai 2011a, b). Studies of simulation models related to soil moisture (Wang 2005; Sheffield and Wood 2008), drought indices (Dai 2011a; Rind *et al.* 1990; Burke and Brown 2008) and precipitation-minus-evaporation (Seager *et al.* 2007) indicated increased incidence of water stress in future (Minhas *et al.* 2017). Previous climate model studies (Giannini *et al.* 2003; Schubert *et al.* 2004; Seager *et al.* 2005; Hoerling *et al.* 2006; Schubert *et al.* 2009) showed that differences in sea surface temperatures have a large impact on precipitation. The inability of the coupled model simulations to reproduce observed regional precipitation changes, was linked to natural change patterns in sea surface temperatures (Hoerling *et al.* 2010). It is concluded that the observed global aridity changes up to 2010 are consistent with model predictions, which suggested widespread and drastic drought incidence in the coming 30–90 years over many land areas with follow-on reduced precipitation and/or increased evaporation.

2. Origin of phenomics

Continuously growing populations and escalated food requirements to feed the increasing population are serious concerns for farmers and agriculture scientists. According to the reports of FAO (2009), it was estimated that almost 70% more food production is a requisite globally to feed an additional 2.3 billion population by the year 2050 and this is one of humanity's greatest challenges. The recent advances in plant phenotyping techniques and DNA sequencing, in concert with large data sets analysis, have given rise to 'phenomics'. Phenomics refers to the total of all phenotypes at different levels, ranging from molecule to organ level. This term also pertains to whole-organism study in terms of growth, performance, composition, architecture, data acquisition, using high-throughput phenotyping methods and data analysis (figure 1). The term phenomics was given by Gerlai, in the year 2002, to imaging techniques that allow researchers and scientist to learn the obtain information about plants at their root or whole-plant levels and the inner working mechanisms of leaves. Phenomics includes techniques such as infrared imaging, 3D imaging, magnetic resonance imaging, fluorescence imaging and spectral reflectance.

3. Forward and reverse phenomics

Phenotyping is very important for the production of elite plants, for functional analysis of specific genes, and for forward and reverse genetic analysis. High-throughput phenotyping is also necessary for phenotyping of many different lines such as germplasm collection, breeding population, mapping population, mutant populations, under varied growth conditions. Plant phenotype is an inherent complex interaction of genotype with its definite environmental conditions that influence the growth and development of plants. According to Tardieu and Tuberosa (2010), plant phenotype can be described by means of structural traits, while plant functioning described by means of physiological traits.

Forward phenomics uses phenotyping tools to distinguish most promising genotypes having the most desirable traits within a large collection and this allows selection of the 'best of the best' genotype/elite line. Thus, it enables rapid identification of traits at pre-stage and makes it less necessary to grow plants up to the maturity stage in field (Kumar *et al.* 2015). The application of 'forward phenomics' facilitates the screening of a large number of pots (containing plants) that run on a conveyor belt and pass through a chamber comprising automated imaging systems (Tsaftaris and Noutsos 2009). Studies were also done in many crops like rice, wheat, sorghum, barley, *Brassica*, and *Arabidopsis* (Yang *et al.* 2020) for trait-specific phenotypes. These studies also enable comparison of the traditional and phenomics approaches to find the effectiveness of symbiotic association among the *Bradyrhizobia* strains with soybean under drought stress (Govindasamy *et al.* 2017).

On the other hand, after the 'best of the best' genotype is identified by using forward genetics, reverse genetics allows in-depth mechanistic understanding of traits shown to be valuable. Further, the identified mechanisms are used to exploit new approaches (Kumar *et al.* 2015), and therefore, reverse

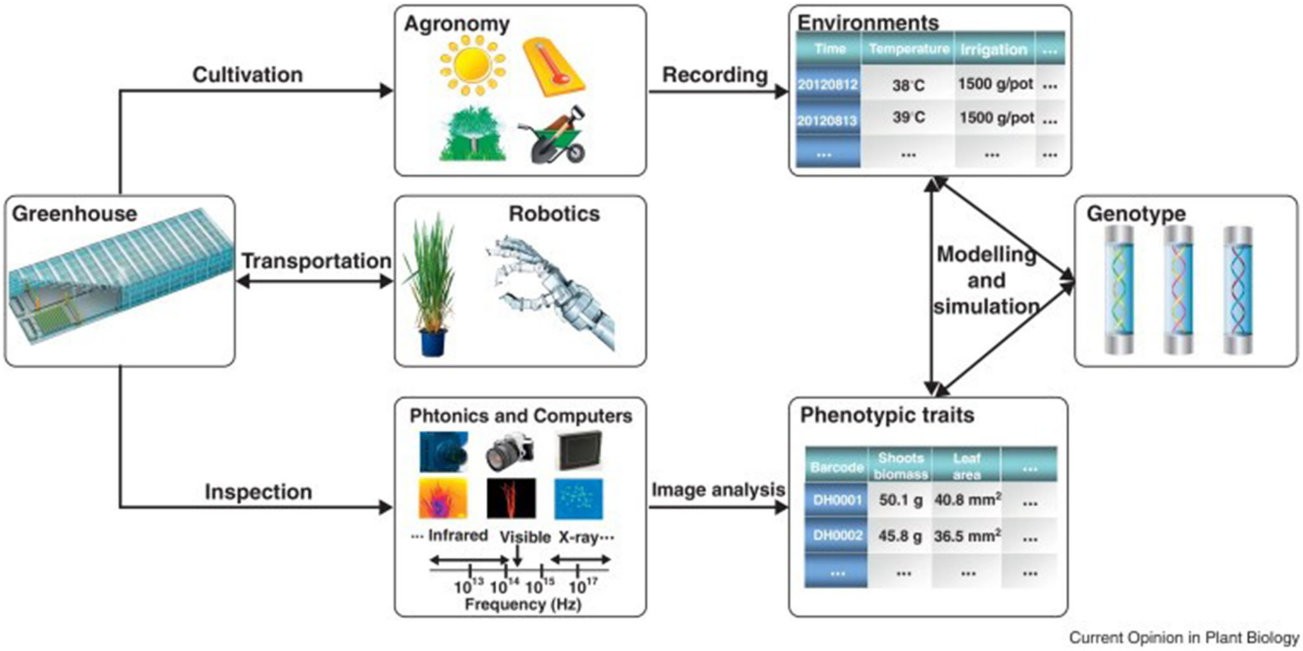

Figure 1. Overview on plant phenomics techniques (plant phenotyping platforms). The rice plants screened by using robotics and are transported to inspection chamber. The inspection chamber consisted of plant phenotyping platforms and plant's traits are screened (non-invasive) using computers and photonics. Subsequent to image analysis, quantified traits and environmental data together are managed in database that provides a 'phenotype–genotype model'. The model facilitates simulation or predication of responses for each genotype in varied environmental conditions (*Source*: Yang *et al.* 2013).

genetics helps in making 'best' varieties the superior traits/genes. This involves the conversion of the physiological level to the biochemical and or subsequent molecular level of a trait or gene. For example, researchers identify the mechanism involved in drought tolerance followed by a gene or a group of genes which is responsible for tolerance (Kumar *et al.* 2015). Plant phenomics includes a series of high-throughput screens, multiple camera units, non-destructive measurements, quantitative analysis, monitor growth dynamics, and stress assessment link to genomics, opening new prospects (Chen *et al.* 2014; Li *et al.* 2014).

4. Advances in phenomics

High-throughput plant phenomics includes imaging techniques that allow phenotyping of many plant populations within a short time at each plant level. Some phenomics high-throughput techniques (Yang *et al.* 2020) are 3D imaging, infrared imaging, fluorescence imaging, visible light scanning and magnetic resonance (Sozzani *et al.* 2014). In 3D imaging techniques, pots containing plants travel on a conveyor system through an imaging chamber (Tsaftaris and Noutsos 2009), and 3D models are generated automatically in a computer. Thermal infrared cameras use light in the far-infrared region of the spectrum between 15 and 1000 μm to study plant canopy temperatures (Nasarudin and Helmi 2011). The variation in temperature will further help in studying photosynthesis efficiency, salinity and drought tolerance (Jones *et al.* 2009; Munns *et al.* 2010). A fluorescence image appears when an object emits light of certain wavelength while absorbing light of a different wavelength. This technique allows measurements of the photosynthesis process and plant health. In this regard, to study the influence of different genes or environmental conditions on photosynthesis efficiency, chlorophyll fluorescence is used (Schreiber 1986; Daley *et al.* 1989; Maxwell and Johnson 2000; Baker 2008). In visible light scanning, a variation in color gives an estimate of the plant/leaf senescence. The senescence of matured leaves reflects escape or avoidance mechanisms, which is adopted by the plant under water stress conditions, whereas genotypes with stay-green can continue the photosynthesis process under water stress and are identified as tolerant (Howarth *et al.* 2011). Magnetic resonance imaging (MRI) is an imaging technique which is frequently used to study plant roots. The root images are captured in the same way as imaging body organs in medicine by using a magnetic field and radio waves.

MRI facilitates 3D geometry of roots that are exactly similar to the actual plant growing in soil. The spectral reflectance is the fraction of light reflected by a non-transparent surface (Jahnke *et al.* 2009; Borisjuk *et al.* 2012). Researchers can use spectral reflectance to identify a plant that has suffered from salinity or drought prior to the appearance of symptoms. A hyper-spectral camera measures all wavelengths of light that are either reflected or absorbed by a plant (Chen *et al.* 2014).

5. Relevance of high-throughput phenomics

5.1 *Identification of stress*

Drought-tolerant wheat crops are used with different amount of water at various growth stages under different environmental conditions. To breed drought-tolerant wheat, researchers have to study the performance of crops in the field over a whole growing season. Phenomics remote sensing technology can measures plant growth, canopy temperature and other traits under drought stress conditions. Similarly, the researchers from Commonwealth Scientific and Industrial Research Organisation (CSIRO) screened wheat and barley crop that were grown in saline conditions and tolerant varieties were identified by using phenomics approaches (Berger *et al.* 2010; Munns *et al.* 2010; Chen *et al.* 2014).

5.2 *Study of various physiological processes*

The supercharging photosynthesis plants have two major photosynthetic mechanisms, i.e., C_3 and C_4. Phenomics researchers want to replace the C_3 pathway of rice with a more efficient C_4 mechanism. The C_4 plants can concentrate carbon dioxide inside the leaf and photosynthesize more efficiently compared to C_3 plants (von Caemmerer *et al.* 2012). A major limiting factor in photosynthetic performance is the inefficiency of the enzyme Rubisco. Some plants have better Rubisco efficiency than others. Using phenomics, researchers are searching through thousands of wheat varieties with a better performing Rubisco and higher rates of photosynthesis that can grow well under nutrient deficiency, drought and salinity (Tackenberg 2007; Baker 2008).

5.3 *Rapid and efficient screening for mutants*

In the field of phenomics, remote sensing technology enables plant researchers to study a large number of plants in field conditions. Measurements can be taken on many plants at once and over a whole growing season. Some examples of phenomics field technology are phenonet sensor network, phenomobile, phenotower and multicopter. These technologies were also used in detection and monitoring of disease epidemics in the field and root attack by pathogens, facilitating the screening of germplasm and modeling of biomass production (Miyao *et al.* 2007).

6. Conclusion and future directions

Phenomics imaging techniques are essential for functional analysis such as identification, study of the processes/mechanism, rapid and efficient screening, dissection and confirmation of specific genes, forward and reverse genetic analysis, and for production of elite plants. Most of the available plant phenomics technologies are confined to controlled conditions, which is a constraint for generating phenotypic data in a changing micro-climate. Perhaps, field phenomics imaging tools will move on to standing crop germplasm/breeding lines/mutant populations in the field for generating realistic, precise, multi-dimensional and robust phenotypic data. Recently, some robust field imaging technology sensors, such as phenonet sensor network, phenomobile, phenotower and multicopter, were employed for realistic data generation. The cost-effective, user-friendly technology attached with simple image processing software and easy statistical analysis programs is necessary, for increased utilization of these plant phenomics technologies to identify the climate-resilient varieties.

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