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# Plant Phenomics: Bridging the gap between Plant Physiology and Genetics

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**Abstract:** As area of biology concern with measurement of phenomes, the physical and biochemical traits of plants ,phenomics allow to perform pharmaceutical research and it also used in functional genomics and in metabolic engineering. The use of molecular marker and mapping populationsin combination with the array based identification of gene regulation mechanism has led to the accumulation of an unprecedented amount of genetic data.Basic requirements of an ideal phenomics effort are easy to state but difficult to achieve: genomic information on a large sample of genotypes, which are each exposed to a range of environments; extensive and intensive phenotyping across the full range of spatial and temporal scalesMordern phenotyping methods are used recently as described below.A major goal of plant phenomics is to develop high throughput phenotyping methods for elucidating changes in the behaviour of the root system with changing environmental conditions.

**Key words:**infrared,Carbon isotope discrimination, Phenomics Ontology Driven Database,Chlorophyll fluorescence,Thermal Infra-red imaging

## I. INTRODUCTION

Population of earth continuing to increase rapidly, estimated to reach 9 billion by 2050. It has been calculated that in order to meet the increasing agricultural demand, there must be increase in global production of 44 million tonnes per year which is considerably more than current average increase of 32 million tonnes per year (Tester and Langridge, 2010). In addition to global population increase, one of the serious concernsto agricultural productivity is the global environmental change such as abiotic stresses, climate change, land degradation and declining water quality (Tester and Langridge, 2010). These abiotic stresses are often complex phenomenon, and adaptation of plant to these abiotic stresses involves range of traits (Roy et al., 2011).

Modern genetic approaches involve identification of genes involved in tolerance to environmental stresses, and being engineered to confer abiotic stress tolerance in crop plants under laboratory conditions. For most cases, it is not going to be a simple matter of identifying a gene that will provide resistance to one abiotic stress, leaving all other such stresses in environment. Thus the complexity of plant stress adaptation make breeding and reverse genetics based approaches for abiotic stress tolerance, particularly drought and salt complicated (Roy et al., 2011). Therefore, a more powerful approach to identify naturally occurring variations of abiotic stress tolerance in varieties, landraces and wild relatives of crops, and to study the traits that contribute to tolerance. In order to incorporate these traits into molecular breeding programmes and to identify the underlying stress related genes in a forward genetic approach, a reliable phenotyping protocols are extremely important (Salekdeh et al., 2009). Advent of high throughput techniques have made gene and marker identification faster than ever (Berger et al., 2010). With the availability of the genetic resources such as mutant population or mapping populations, high throughput phenotyping will be essential to close the gap between plant physiology and genetics (Finkel, 2009).

Conventional methods for phenotyping plants are frequently laborious, destructive and often involve plant biomass for analysis.Recent development in the high throughput, non-destructive imaging technologies allows the researcher to obtain multiple images of the same plant at different time points and different wavelength. Therefore, offering new, non-destructive methods for acquiring quantitative data on plant growth, health, and water use under abiotic stress (Jones et al., 2009; Munns

 

et al., 2010; Berger et al., 2010). With advent of these modern technologies, a new omics approach for the crop improvement has been developed, namely, 'Phenomics'.

Plant Phenomics can be defined as simply "High through-put plant physiology" or "Acquisition of three dimensional phenotypic data on an organism wide scale" (Furbank and Tester, 2011). Plant phenomics is the study of plant growth, performance and composition. Forward phenomics uses phenotyping tools to 'sieve' collections of germplasm for valuable traits. The sieve or screen could be high-throughput and fully automated and low resolution, followed by higher-resolution, lower-throughput measurements. Screens might include abiotic or biotic stress challenges and must be reproducible and of physiological relevance. Reverse phenomics is the detailed dissection of traits shown to be of value to reveal mechanistic understanding and allow exploitation of this mechanism in new approaches. This can involve reduction of a physiological trait to biochemical or biophysical processes and ultimately a gene or genes (Roy et al., 2011). Using phenomics based approach; plant performance in field under various stresses can be evaluated much faster and more dynamically, involving complete lifecycle measurement which is less dependent on periodic destructive assays. Furthermore, application of these tools in dedicated high throughput, controlled-environment facilities has the potential to improve precision and reduce the need for replication in the field. This brings us to the age of 'phenomics' (Furbank and Tester, 2011).

**Genomics versus Phenomics**

Phenomics is defined in analogy with genomics, but this comparison is misleading in one aspect that we can completely characterise a genome but not a phenome. This is because phenotypes vary from cell to cell and from moment to moment depending upon the external environmental factor (Houle et al., 2010).

**Why Phenomics?**

**Studying the genotype-phenotype map**

The concept of a genotype–phenotype map is a widely used explains the multiple ways in which genotypic information influences the phenotype of an organism. Phenomics is most frequently justified as enabling us to trace causal relationship between genotype, environment and phenotype (Houle et al., 2010). To harness the wealth of genomic information for agricultural application, it has to be carefully and comprehensively relate the phenotype in a real world environment (Furbank and Tester, 2011). Lein et el., 2008 carried out high throuput gene silencing and visual phenotyping of transgenic tobacco, to identify genes in which partial inhibition of expression leads to marked phenotypic changes mostly on leaves.This procedure is more stringent than the analysis of knockout mutants, because it requires that even a partial decrease in expression generates a phenotype.This procedure identified 88 validated gene/phenotype relations. These included several previously characterized gene- phenotype relationships, demonstrating the validity of the approach.

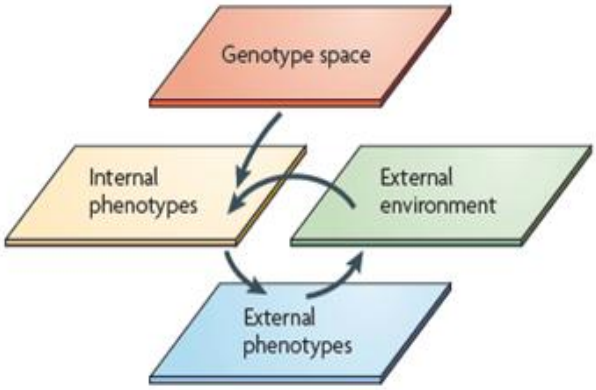


 

**Fig.1 The genotype-phenotype map (**houle et al 2010**)**

**Identifying genetic basis of complex traits**

High throughput phenotyping based rapid marker generation and gene identification will accelerate the identification of traits and genome loci, contribute to stress tolerance in plants(Munns et al., 2010). Kuromori et al., 2009 published detailed investigation on phenome analysis in plants using loss-of-function and gain-of-function mutants. Zhang et al., 2012 highlights a cost-effective, high-throughput phenotyping approach that facilitates the dissection of genetic basis of shoot growth and development under dynamic environmental conditions in *Arabidopsisthaliana*. Karsai et al., 2008 using various high throughput phenotyping methods compare effect of photo and thermal cycle on the flowering time in barley.

**Causal explanations at the phenotype level**

The simplest justification for phenomics is that the characteristics of organisms of greatest interest to most biologists are phenotypes rather than genotypes such as yield, efficiency, resistance and adaptability of the plant under stress condition. In future, there is need to explain why phenotype vary in a population between species. Using various phenotypic methods such as stomatal conductance, metabolite profiling, osmolyte accumulation, canopy temperature chlorophyll fluorescence and water use efficiency, researchers can predict about the adaptability of cultivars that can tolerate the harsh environmental conditions (Roy et al., 2011).

**Mordern phenotyping methods and their underlying principles**

**Thermal Infra-red imaging**

The integrator of drought is the plant water status, as determined by plant water content or water potential. It is the result of equilibrium between root water uptakes and shoot transpiration (Jones, 2007). Direct measurement of these variables is very difficult by traditional methods (Blum et al., 1982). Currently, a high throughput technique, Thermal Infra Red Imaging or Infra Red Imaging is used to measure leaf or canopy temperature (Jones et al., 2009). IR thermography 'visualises' surfacetemperature distribution of an object by focusing the longwaveradiation emitted by the object onto a temperaturesensitivedetector – the object's temperature determines howmuch radiation is emitted at what wavelength followingPlanck's law. Evaporation is commonly the main determinant of leaf temperature due to its large cooling effect and there is direct relationship between leaf temperature, transpiration rate and stomatal conductance (Sirault et al., 2009). In the study carried out by Lu et al., 2008 reported that a cooler canopy for stomata and higher transpiration rate account for improved yields as there was no apparent change in photosynthesis. Similarly, Fischer et al., 1998 reported the relationship between higher stomatal conductance, cooler canopies and increased photosynthesis leading to higher yield in wheat and barley in water limiting environment. Sirault et al., 2009 showed that leaf temperature between barley grown at 0 and 200 mM NaCl reached about 1.6 ºC using IR thermography for the screening of tolerance to osmotic stress in cereals, suggesting that under stress, leaf temperature increases due to imbalance between water uptake and transpiration. James et al., 2008 and Qiu et al., 2009 supported the potential of IR imaging for screening a large number of genotype varying for stomatal traits under stress conditions. IR phenotyping methods acquires more precise measurements in a fraction of time needed to perform several replicate reading per plot to the changing environmental conditions between measurement with help of infra red thermometers and cameras, and also colour images have been used successfully to identify sunlit and shaded leaves in a canopy, helps in determining the respective leaf temperature (Berger et al., 2010). There are some drawbacks in IR imaging such as separation of the plant canopy of interest from background soil (which is hotter than leaves).Wide range of leaf temperatures, resulting from both variations in stomatal conductance through canopy and variations in observed short wave solar radiation depending on leaf and solar orientation. Temporal variation resulting from non constant illuminations such as cloudy days (Furbank and Tester, 2011)

 

**Visible and Near Infra Red Imaging**

Solar radiation occurs predominantly in the spectral range of the visible and near infra red regions, with wavelength ranging from 400nm to 2500nm. The interaction of solar radiation with plant has been a major focus in remote sensing and in attempt to monitor the stress level of vegetation. Hyperspectral reflectance measurements (progressive scanning of very narrow wavebands) have enabled the identification of spectral indices representing the plant stress levels.In the visible spectrum, reflectance by single leaves orcanopies is particularly low. This is due to absorption by leafpigments, mainly chlorophyll, with a characteristic peak of reflectance in the green region of around 550 nm. With the transition from the visible to the NIR wavelengths, there is a sharp increase in reflectance, the so-called 'red edge'. In the NIR, between 800 nm and 1300 nm, a large proportion ofthe incident radiation is reflected by leaves due to scattering within the leaf mesophyll (Knipling, 1970). NIR radiation can be transmitted from the upper leaves of the canopy to the lower leaves, which can reflect the photons back to the upper part of the canopy. As a consequence, leaf and canopy architecture, such as leaf thickness and growth habit, are the major determinants of the reflectance pattern in this part of the spectrum. With increasing wavelengths, beyond 1300 nm, reflectance decreases gradually due to an increased absorption by water present in the leaves with characteristic water absorption bands at 1450 nm, 1930 nm,and 2500 nm (Knipling, 1970): this is the basis for using NIR imaging to study water stress of plants (Eitel et al., 2006; Seelig et al., 2008).In field condition, greenness and biomass accumulation was estimated using reflectance measurement in visible and NIR wave range (Harris et al., 2007). One of the major advantages of Visible and NIR imaging is easy to use and have wide application in assessment of plant health in response to stress condition such drought, salinity or nutrient efficiency (Peneuelas et al., 1998; Perry and Roberts 2008). Imaging has an advantage that it can resolve heterogeneity occurring at canopy, plant or leaf level. Imagings also make it possible to separate plants from background soil, which is important in early and late stage of growth when plant do not entirely cover the ground. While drawbacks related to Visible and NIR imaging are 1) Hyperspectral reflectance measurements are not feasible for high throughput approach due to the time needed to acquire stable spectra and 2) Incident sunlight is greatly influenced by atmospheric water vapour at about same wavelength leading to an extremely high noise to signal ratio. (Berger et al., 2010).

**Chlorophyll fluorescence analysis**

Imaging of chlorophyll fluorescence is used as a diagnostic tool in many areas of plant physiology (Baker, 2008), such as the early detection of stress symptoms induced by pathogen attack or herbicide treatment, where a spatial resolution of the heterogeneity is important (Konishi et al., 2009).Chlorophyll fluorescence has also been used as a surrogate measurement for maintenance of photosynthetic function under stresses such as drought (Woo et al., 2008). The most easily measured, and hence the most commonly used, fluorescence parameter in stress studies is dark-adapted Fv/Fm, a measure of the intrinsic photochemical efficiency of light harvesting in photosystem II (Baker, 2008). This measurement is now possible using affordable commercial instrumentation designed for imaging of whole leaves or small plants using pulse amplitude- modulated (PAM), fluorometry [Baker, 2008]. It is feasible in high-throughput to obtain whole-plant average measurements or to target leaves at the same developmental stage if the commercial systems and software are adapted to this purpose. This is particularly applicable to high-throughput studies of stress response in species which grow predominantly in the horizontal plane in the seedling stage. Fluorescence imaging also allows the determination of projected leaf area and hence the growth rate if measurements are made regularly over time (Barbagallo et al., 2003).Fv/Fm has recently been measured in two drought studies with Arabidopsis using systems scalable to high-throughput screening (Woo et al., 2008; Jansen et al., 2009). Rolfe and Scholes (2009) studied use of chlorophyll fluorescence imaging in plant pathogen interaction as a method for the early detection of viral, bacterial and fungal infection, before symptoms are visible by eye, and also as a means with which to probe underlying pathogen-induced changes in host physiology in both compatible and incompatible interactions.

**Carbon Isotope Discrimination (CID)**

 

Carbon isotope discrimination (termed CID in plant breeding) as a reproducible indicator of transpiration efficiency in crop physiology and plant breeding (Richards et al., 2010). This technique is derived from the observation that plants discriminate between against the heavy isotope of carbon ($^{13}C$) naturally present in atmospheric $CO_2$, both in the process of $CO_2$ diffusion into the leaf and in the metabolic processes of photosynthesis (Codon et al., 2004). This isotopic discrimination is reflected in the isotopic signature of plant dry matter. Richards et al., 2010 showed that in $C_3$ crops, CID values are strongly related to stomatal conductance and transpiration efficiency for a given photosynthetic capacity. In addition to this, this technique used to find genetic variation in transpiration efficiency in wheat and to breed commercial varieties with greater water-use efficiency and yield (Richards et al., 2010). The utility of this approach is that samples can be collected at the end of the growing season and the isotopic composition reflects the integrated effect of the entire growing season, avoiding the issues of measuring leaves and plant organs at key phenological stages. One of the main drawback of CID is that its high cost per sample are approximately US$30 per sample and the need to normalize datato photosynthetic capacity or yield potential to obtain varieties which are both good performers in terms of growth and yield and conservative consumers of water. It is not known whether CID measurements can be scaled up to the field from seedling measurements in controlled environments (Furbank and Tester, 2011).

**Phenomics Databases**

High-throughput phenotyping of model plants such as Arabidopsis using non-invasive imaging technologies is a rapidly advancing field (www.plantphenomics.org.au) and Image analysis and mathematical treatment ofimaging data to extract growth dynamics, morphologicalcharacters, and spatially described photosynthetic parametersare challenging and require sophisticated storageand linkage of primary images and calculated data. Hence there is a necessity of developing databases of phenomics information requires comprehensive metadata description and agreed ontologies. Well-described metadata mitigates the issue of standardized experimental conditions to some degree and recently there have been two attempts to provide ontology-based solutions to combining metadata repositories with phenotypic databases and search tools (Furbank and Tester, 2011). After the whole genome sequencing, it is quiet easier to validate the phenotypic expression of genes. Kuromori et al., (2006, 2009) carried out a study that provides basic data on large-scale phenotyping of gene knockout lines in plants, and will contribute to the completion of an international effort to develop aphenome database of all the functional genes in Arabidopsis. Australia is leading the way in developing Phenomics Ontology Driven Database (PODD) (http://www.plantphenomics. org.au/ PODDProject) at the Australian Plant Phenomics Facility (APPF). PODD is intended to service both plant and animal phenomics focusing on metadata and ontologies linked to a webbased graphical user interface, it utilizes different software solutions to achieve these aims. For plant applications, it is intended to provide a mechanism for archiving and retrieval of phenotypic data produced from imaging, spectral analysis and a vast array of physiological (phenomics) data produced from the Australian Plant Phenomics Facility (www.plantphenomics.org.au).

**Goals and Technical Challanges of Phenomics**

Current phenomics efforts largely adopt extensive sampling by choosing a wide range of conventional, low-dimensional measurements. The first priority of a phenome project would be to develop technologies that maximize throughput phenotypingand substantially lower the cost of doing so. Funding through a coordinated programme designed to further the goals of phenomics could help in the development of technologies for generalization include metabolomics, imaging, microfluidics and nanotechnology. Increasing in the quantitative information obtained by phenotypic measurements is another important goal for phenomics (Houle e al., 2010). Phenomic data raises the possibility of addressing the ‘many to many’ relationship that is inherent with the interaction of genotype and phenotype. Plant phenomics lack techniques to deal with such huge data. So there is current challenge to international researchers to develop a web based repository of phenotypic data (Furbank, 2009) and phenotypic data is being shared and made commonly available. A non-expert user will need to understand and be able to interpret image-derived phenotypic data and be able to access this information without prior knowledge of image analysis. Hence, the generation of publicly available databases is important and they have to be carefully designed to be useful for the broader plant science community. A major goal of plant phenomics is to develop high throughput phenotyping

 

methods for elucidating changes in the behaviour of the root system with changing environmental conditions. A few reports have been published in advancement in phenotyping of roots (Yazdanbakhsh and Fisahn, 2009). In addition to this we have to develop more efficient method to control the environmental factors while recording the phenotypic data.

**Future of Phenomics:**

The basic requirements of an ideal phenomics effort are easy to state but difficult to achieve: genomic information on a large sample of genotypes, which are each exposed to a range of environments; extensive and intensive phenotyping across the full range of spatial and temporal scales; and low cost. A $40 million venture of Australian Plant Phenomics Facility (APPF) headed by Mark Tester consisting of two nodes, one is High Resolution Plant Phenomics Centre (HRPPC) in Canberra, an international collaboration to screen *Brachypodium* variants for drought-tolerance and for less lignin in their cell walls and The Plant Accelerator in Adelaide (Finkel, 2009). It is clear that the cost of a phenome project using current technology would be extremely high and we see the attractiveness of a phenome project. The current development in plant phenomics boost researcher's effort to replace rice inefficient C3 photosynthetic pathway with C4 pathway found in maize and 40 other plant species. A major challenge in achieving this objective is identifying and engineering the genes necessary to install $C_4$ photosynthesis in rice. An international research consortium was established to achieve this aim. Central to the aims of this project is phenotyping large populations of rice and sorghum (*Sorghum bicolor* L.) mutants for '$C_4$-ness' to identify $C_3$ plants that have acquired $C_4$ characteristics or revertant $C_4$ plants that have lost them (Furbank et al., 2009).Phenomics tools can provide snapshots of cellular structure and diagnose steps along the way toward C4 metabolism in live plants.

**Conclusion**

The comprehensive nature of genomic data has spawned entirely new disciplines that use the availability of genome sequence as a starting point. By identifying phenomics as a discipline inits own right, we can accelerate progress in the parts of plant phenomics that have been benefited only indirectlyfrom genomics**.**Abiotic stress tolerance is complex, but as phenotyping technologies improve, components that contribute to abiotic stress tolerance can be easily quantified. Plant phenomics can, in fact, be considered as simply plant physiology in 'new clothes', but it promises to bring physiology up to speed with genomics by introducing the incredible recent advances made in computing, robotics, machine vision and image analysis to the wider field of plant biology. A multidisciplinary team in plant phenomics crosses biology, physics and mathematics, not 'just' genetics, biochemistry, physiology and plant breeding. This trans-disciplinary approach promises significant new breakthroughs in plant science. Phenomics provides the opportunity to study previously unexplored areas of plant science, and it provides the opportunity to bring together genetics and physiology to reveal the molecular genetic basis of a wide range of previously intractable plant processes.

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