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Imaging techniques to assess plant responses to abiotic stresses

Mahesh Kumar*[1] Jagadish Rane[1], Narendra Pratap Singh[1] and Mukesh Kumar[2]
[1]ICAR-National Institute of Abiotic Stress Management, Baramati, Pune-413115 (Maharashtra), INDIA
[2]Department of Seed Science and Technology, Bihar Agricultural University, Sabour, Bhagalpur-813210 (Bihar), INDIA
*Corresponding author. E-mail: mahesagrawal@gmail.com

ABSTRACT

To ensure food demand which required satisfying the needs of a human population that isexpected to grow to more than 9 billion by 2050 is a terrific challenge. To bridge the gap, high yielding, stress-tolerant plants can be selected more rapidly and efficiently than is currently possible. Precise and accurate measurement of traits plays an important role in the genetic improvement of crop plants. Lot of development has taken place in the area of phenotyping in the recent past. Effective, high-throughput phenotyping platforms have recently been developed to solve this problem. A variety of imaging methodologies include visible imaging, thermal imaging, fluorescence imaging eta are being used to collect data for quantitative studies of complex traits related to the growth, yield and adaptation to abiotic stresses. Here we provide an overview of image based phenotyping techniques people using for abiotic stress study.

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INTRODUCTION

Drought has been a recurring feature of agriculture in India [1] and it occurs over an extended period of time and space, making it unpredictable and the losses are not quantifiable easily. But the impact of drought on the techno-economic and socio-economic aspects of agricultural development and growth of the nation is severe and results in huge production and monetary losses. During the period 1900–2014, the number of occasions on which large Indian population got affected from drought was more than any other natural disaster. Occurrence of drought is very frequent in the meteorological subdivisions like Maharashtra, West Rajasthan, Tamil Nadu, Jammu and Kashmir, and Telangana. The risk involved in successful cultivation of crops depends on the nature of drought (chronic and contingent), its duration, frequency and timing of occurrence within the season and the soil type.

It is increasingly evident that the gains in agricultural output provided by the green revolution have reached their ceiling whereas the world population is expected to reach nearly nine billion by 2050. The recent plateau in genetic gain in productivity of crop also indicates that possibly we are at attainable maximum productivity of crops with traditional method of crop improvement even with all the favourable factors for crop growth in place for high productivity zones. Therefore in addition to increasing the yield of crop plants in normal soils, there is an absolute need to enhance productivity and stability of crop yield in less productive lands, including salt affected lands. This is more relevant to highly populated countries like India where an estimated 6 to 7 million ha land is affected by salinity/alkalinity and about 2.0 million ha of salt affected land is being reclaimed. Further, it is being predicted that salt affected area is likely to increase to an extent of 16.2 million ha by 2050 mainly due to expansion in irrigated area, intensive use of natural resources responsible for second generation problems and also due to predicted climate change.

In this context, there is a need for concerted effort to improve tolerance to drought, high temperature and salinity are to be incorporated through genetic improvement. This needs suitable traits for introduction into the existing cultivars and we have to search for source of such traits and genetic variability existing for this trait. This is prerequisite for identification of genes associated with these traits that contribute to stress tolerance. Though this approach is not new, the advances in genomics have added new dimension to this approach for enhancing our capacity to develop new cultivars with stress tolerance. Much of these advances is apparent in enhanced capacity to understand genes in crop plants. However, the characterisation of plant responses to stresses can greatly complement genomic efforts.

Destructive phenotyping methods that include harvesting plant responses for assessment of water relations and other physiological responses to stresses limit our studies to very few plants and make this exercise cost and labour intensive. Hence, in the first generation of instrumentation for non-invasive studies several equipments such as photosynthesis meters, stomatal conductance meter, SPAD meter, chlorophyll fluorescence meter, NDVI sensors emerged as handy tools for physiologists, breeders and agronomists for field studies. The current phenotyping platforms include a variety of imaging methodologies to obtain high-throughput non-destructive phenotype data for quantitative studies of complex traits, such as growth, tolerance, resistance, architecture, physiology, yield, and the basic measurement of individual quantitative parameters that form the basis for more complex traits [2,3]. Here, an attempt has been made to focus on non-invasive methods which use images for assessing plant responses. These methods are based on images captured by background system that senses different bands of wavelength in electromagnetic spectrum. They include visible, infrared, fluorescence, NIR/SWIR, hyper spectral/multispectral *etc*.

Imaging systems

: Colour, morphology, geometry	Visible
: Canopy temperature	Infrared
: Efficiency of photosystem	Fluorescence
: Water content, thickness	NIR/SWIR
: Spectral stress indices	Hyper spectral/ multispectral

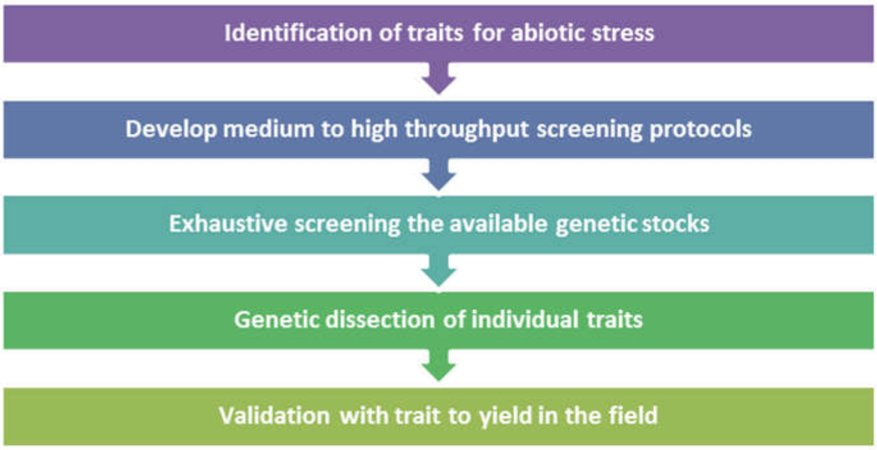

Fig. 1. Application of non-destructive phenotyping in genetic dissection of trait

Imaging Devices for Phenotyping

Visible Light Imaging

A visible image is intended to mimic human perception to provide information or input to systems that need data for plant phenotyping applications to trait-based physiological breeding.In plant science, visible light imaging has been broadly adopted due to its low cost and simplicity. Using this imaging system, with a similar wavelength (ranging from 400 to 700 nm) perception as the human eye, two-dimensional (2D) images can be used to analyze numerous phenotypic characteristics and to record the changes in plant's biomass [4]. To spread the spatial and volumetric information of phenotype images, three-dimensional (3D) imaging approaches have been developed, which could provide more accurate estimations of the morphological features [5].

Therefore, during the integration of 2D and 3D image analysis, visible light imaging techniques are popular components for the integrated plant phenotyping platform [6]. It represents raw data of a phenotype image in spatial matrices based on the intensity values relating to photon fluxes (red~600 nm, green~550 nm, blue~450 nm) of the visible light spectral band. In the field, visible images provide information on the canopy arctitecture, canopy cover and canopy color, leaf areaindex (LAI), can be obtained with this method [7].Although, it is the most trivial method in plant phenotyping, the drawback is that visible images only provide physiological information, and the common problem is created by the overlapping adjacent leaves and soil background during segmentation process [3]

Infrared Imaging

The surface temperature of canopy is associated with the amount of transpiration results in evaporative cooling. CT used routinely, particularly for stress diagnostic and breeding selection of stress adapted genotypes: (i) under drought conditions it is related to the capacity to extract water from deeper soil profiles and/or agronomic water use efficiency (WUE); (ii) under irrigated conditions it may indicate photosynthetic capacity, sink strength and/or vascular capacity –depending on the genetic background, environment and developmental stage; and (iii) under heat stress conditions is related to vascular capacity, cooling mechanism and heat adaptation. Infrared radiation is also a kind of electromagnetic radiation like visible light, which measures the surface temperature of any objects. Generally, temperatures measured are proportionate to amount of infrared radiation emitted from the surface. With same principle, recently infrared imaging systems have been developed and are engaged in assessing the canopy temperatures of plants. Infra-red thermometry or IR thermography measures temperature of the target by measuring the radiant thermal energy emitted by the target. Infrared is a type of electromagnetic radiation, which is emitted, to greater or lesser degree, by all objects that have temperature. IR spectral region of 8 to 13 µm is typically used for thermal remote sensing. Infrared imaging technologies are used for screening objects of internal molecular movements which emit infrared radiation. Two popular infrared imaging devices- a near-infrared (NIR) and a far-infrared (Far-IR, also called IR thermal) - can be used to screen radiation images. Many studies have combined visible and NIR imaging to detect vegetative indices due to the fact that healthy plants reflect a large proportion of NIR light (800–1400 nm), whereas soil reflects little NIR light. Moreover, soil and unhealthy plants reflect considerably more red light as compared with healthy plants.

The major advantage of visible light and NIR imaging are that they can assess plant health status response to different stress conditions. Visible and NIR digital imaging techniques are more suitable for screening multi-traits and nitrogen status under stress condition [8]. For drought resistance, IR thermal imaging can be used to visualize temperature differences. A thermal infrared imaging technique has been introduced in both, laboratories and fields, and can characterize mutant screens, drought tolerance, salinity tolerance, osmotic tolerance, tissue tolerance, and Na^+ exclusion. It can be used to compare chlorophyll pigments, leaf color and canopy temperature. Infrared imaging has improved drought resistance and/or salinity resistance research by quantifying the osmotic tolerance in response to drought or salinity stress.

The benefits of the infrared imaging technologies are that they provide spatial resolution and more precise measurement under changing environmental conditions, and in field trials a large number of plots can be imaged at the same time [3]. One limitation of thermal imaging in the field is that it needs to include correction of soil background, wind impact and effects of transient cloudiness.

IR thermometer Vs Thermal camera

- A IR thermometer gives number whereas, thermal imaging cameras generate an image.
- A IR thermometer reads the temperature of one single spot whereas, a thermal imaging camera gives you temperature readings for each pixel of the entire thermal image.
- Because of advanced optics, thermal imaging cameras can also resolve temperatures from a longer distance. This allows you to quickly inspect

Fluorescence Imaging

Chlorophyll fluorescence is one of the most highly informative, rapid and non-destructive diagnostic methods for the detection and quantification of damage in the photosynthetic apparatus caused by environmental stress. No investigation into the photosynthetic performance of plants under field conditions seems complete without some fluorescence data. Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photo-chemistry), excess energy can be dissipated as heat or it can be re-emitted as light—chlorophyll fluorescence. These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two.

Chlorophyll fluorescence imaging provides information on photosynthetic performance without destruction or contact with the living Plant. It can be used from laboratory to field environment. This imaging technique describes the information about the plant metabolic status that can be obtained by the artificial excitation of the plant photo systems and observation of the relevant responses [3]. The chlorophyll fluorescence involves an emission of red light from chlorophyll pigments that can be used to assess photosynthetic functions, thereby allowing for plant health monitoring [9].

It is based on charge-couple device (CCD) cameras with sensitive fluorescence signals, where the signals occur by illuminating samples with visible or ultraviolet light. There are two types of fluorescence (red to far red region and the blue to green region) generated by the ultraviolet illumination ranging from 340 to 360 nm, and is expressed as a principle of underlying multi color fluorescence imaging. This technique offers the simultaneous capture of fluorescence emission, and provides a quick way to probe photosystem II status *in vivo*[10]. The most common measurement was made with determining the photochemical activity of light harvesting in photosystem II. It was formulated as Fv/Fm.Fv/Fm generally decreases when plants are exposed to stress in the light, and this event provides an easy and fast tool for observing stress [11,12].

There have been several uses of fluorescence imaging proposed for early detection of stress responses to biotic and abiotic factors before a decline in growth can be measured [2,13]. To screen large mutant collections and to characterize mutants with different photosynthetic pigment composition, portable fluorometers, and fluorescence cameras are widely used. It is found useful to measure the photosynthetic efficiency, the electron transportrate and the extent of non-photochemical quenching. The parameters of photochemical and non-photochemical quenching coefficients are used to study Mg deficiency in broad beans[14]. It is also useful for growth traits [15], detect N deficiency in common bean [16] and maize (17], Fe deficiency in cucumber [18], and NUE in maize [19].

Furthermore, fluorescence imaging technique provides powerful diagnostic tool to resolve the heterogeneity problem of leaf photosynthetic performance, and is used in many areas of plant physiology. Most of the fluorescence imaging applications is limited to the seedling level or the single leaves of model crop. However, it is necessary to develop more robust software and standard procedures for the fluorescence image phenotyping, processing, and data analysis.

Spectroscopy Imaging

The use of spectroscopy imaging is very promising for plant phenotyping. It measures the interaction of solar radiation with plants, and originated from remote sensing of vegetation research [3]. Spectral measurements of the electromagnetic spectra can be obtained through multispectral or hyperspectral cameras that are capable of scanning wavebands of interest at high regulation. Multispectral and hyperspectral measurements of the absorption band in the infrared range are used to describe various water statuses that estimate the canopy water content. The best usable examples of spectral measurements is the derivation of a number of reflectance vegetation indices from simple differences between two wavelength reflectance values to normalized reflectance values. The reflected spectra carry the information about plant architecture and health condition, which can be used to evaluate growth characteristics.

Beyond visible and infrared imaging methods, hyperspectral imaging method can divide images into bands, thus providing a huge portion of the electromagnetic spectrum of the images. The high spectral resolution of hyperspectral technologies make it an essential method for detecting the severity of damage caused by insects. The application of spectroscopy imaging is well-suited for field phenotyping when combined with aerial platforms, but the cost of the spectral cameras and its related infrastructures are relatively expensive.

Table 1. Imaging systems for plat phenotyping

Imaging system	Description	Phenotypic trait parameters	Application purpose
Visible light	The visible light imaging technique is camera sensitive and produces gray or color scale images.	Image-based projected biomass, dynamic growth, color, shape descriptors, root architecture, seed morphology, panicle traits, etc.	Plant growth status, biomass accumulation, nutritional status, or health status
Thermal infrared	Thermal imaging sensor includes near-infrared, multispectral line scanning cameras.	Shoot or leaf temperature, surface temperature, leaf and canopy water status, composition parameters for seeds, etc.	Plant temperature responses to the water status and transpiration rate and detect difference in stomatal conductance of the plant for adoption abiotic.
Fluorescence	Detects chlorophyll fluorscence signals using fluorescence cameras.	Photosynthetic performance, quantum yield, non-photochemical quenching, leaf disease severity assessments, leaf health status, etc.	It provides a fleet way to probe photosystem status in vivo, diagnosing early stress responses mapping QTLs for growth-related traits, characterizing mutants with numerous photosynthetic pigment compositions, etc.
Hyperspectral	This imaging technique use hyper spectralcamera, produced continuous, or discrete spectra raw data.	Water content, leaf growth and health status, panicle health status, grain quality, pigment composition, etc.	This imaging technique used to measure spatiotemporal growth patterns during the experiment and provide insight into the diversity of growth dynamics.

Other Imaging techniques

In recent times, modern optical 3D structural tomography and functional imaging techniques have been developed and extended to improve living plant visualization. Functional imaging such as chlorophyll fluorescence imaging and PET (Positron emission tomograpy) are used for finding photosynthetic performance, stress, and focuses on physiological changes. The combination of structural tomography and functional imaging can screen more precise physiological activity of plant. Another novel imaging technique, MRI (magnetic resonance imaging) is used for imaging of internal physiological processes occurring *in vivo*. Screening the dynamic changes in plant functions and structures by the combining technique of MRI and PET provides a novel functional and structural imaging procedure.

The FRET (Förster resonance energy transfer) sensor is another of the non-invasive advanced imaging technologies for high-resolution measurement of small molecules in living tissue based on genetically encoded, ratiometric fluorescent sensors that bind to and report on levels of the target molecule. It is used for molecular phenotyping, and a single FRET sensor can lead to discoveries of multiple pathways and processes involved in the dynamics of the sensor target. The cellular/subcellular location of interest has to be properly characterized and expressed by a FRET sensor, and measurements can be easily acquired with high temporal and spatial resolution. As the application example, FRET has been used in plant tissue to study calcium and zinc dynamics with subcellular spatial and real-time temporal resolution, the characterization of sugar transport in roots of insect seedlings, the identification of novel sugar transporters. To address many basic questions of plant growth and development, FRET could be an outstanding technology for advanced phenotyping.

Each of these digital photonics-based systems acquires phenotype image data from plant laboratories, greenhouse or fields, and monitors these with special imaging sensor via a remote system.

CONCLUSION

Currently, available non-invasive imaging sensors and computer vision approaches are key technologies to quantify plant structure, physiological status, and performance. Use of image base phenotyping method can reduce the time needed for screening-based estimation of growth and development.

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