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HIGH THROUGHPUT PHENOTYPING STRATEGIES FOR SCREENING DROUGHT TOLERANCE IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Rice, which is the staple diet of more than half of the world's population, is known to be sensitive to drought/water-stress affected environment. The increased and recurring incidents of drought around the globe is a serious concern as it threatens people's livelihood by negatively impacting crops and livestock. Increased yield of crops, namely rice, under stress prone conditions is imperative for food security as it contributes immensely to the dietary calorie supply of millions living in poverty in Asia and some parts of Africa. Consequently, this will bank increasingly on the release of cultivars with improved resistance to drought conditions and with high yield stability. As an initiating step, plant phenotyping plays a crucial role in the screening of these cultivars from a vast population, non-invasively and rapidly, and thus, aiming to achieve high sustainability in rice crop production. The novel phenotyping platforms entail: non-invasive sensors, automated data processing to procure phenotypic traits of significance, mechanized delivery of sensors to plants or vice-versa, robotized plant culturing and computerized analysis of processed data in a data management channel. Non-invasive sensors commonly employed in the automated plant phenotyping hinge on spectrometry (Hyperspectral radiometers, FTIR, IR Thermometry, NIR meter, etc.) and spectroscopy (Visual imaging, Hyperspectral imaging, IR thermography, NIR image analysis, Chlorophyll fluorescence imaging, bioluminescence imaging, fluorescence imaging etc.). In this review, the focus is on four classes of pheno-

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typing: (a) RGB (red–green–blue) imaging to measure size, morphology, growth of plants, architecture of the canopies; (b) thermal imaging of plants or canopy to phenotype temperature and other derived indicators (stomatal transpiration or water status); (c) spectral reflectance/fluorescence of leaves, plants or canopies to analyze the pigments and the biophysical and biochemical processes; and (d) root phenotyping to understand physiology and architecture of the root system.

INTRODUCTION

Over half of the world's inhabitants depend on rice for their staple sustenance. Worldwide rice consumption was predicted to attain a historic high of 518.5 million tonnes in 2019-20 (FA0, 2019). It is the essential food crop of Asia, providing over 30 percent of the calories consumed within the region. As compared to the 439 million tons production in 2010, there is an estimated global need for an additional 116 million tons of rice by 2035 (Seck *et. al.*, 2012). It is imperative for food security as rice contributes immensely to the dietary calorific supply of millions living in poverty in Asia and some parts of Africa (Muthayya *et al.*, 2014). Considering the upsurge in population and the associated demand, there is a necessity to increase the yield of rice.

Climate change is an inevitable phenomenon and drought stress is an important abiotic stress affecting rice production (Rejeth *et al.*, 2020). According to the World Health Organization, every year approximately 55 million people around the world are affected by drought stress leading to severe loss to livestock and crops, all around the globe. Drought can be a menace to people's livelihoods, contributes to increased risk of disease and death and incites migration on a large scale. In agriculture, the term "drought" refers to a plight during which the quantity of water available through rainfall and/or irrigation is insufficient to meet the transpiration requirements of the crop. According to Levitt (1972) the different mechanisms or strategies of drought or water resistance can be grouped into two broad classifications: dehydration avoidance and dehydration tolerance. The morphological and physiological features like early flowering, osmotic adjustment (OA), deposition of epicuticular waxes, deep roots, etc., that enable the whole plant, or its parts , to maintain hydration are classified under dehydration avoidance and the characteristics that support the plant to maintain, at least a minimum of, proper functionality in an exceedingly dehydrated state are classified under dehydration (desiccation) tolerance like accumulation of molecular protectants, remobilization of stem water-soluble carbohydrates (WSC), accumulation of molecular protectants (Sheshshayee *et al.*, 2011).

Crops are exposed to the ravages of drought in various ways and to different extents. Regrettably, global climate change will increase the occurrence and severity of drought episodes, notably ascribed to the enhanced evapo-transpiration demand in plants by elevation in temperatures. These variations have already been shown to counterpoise a significant portion of the increase in average yields that arose from technology, CO2 fertilization, and alternate wetting during the past three decades (Lobell *et al.*, 2011; Beena *et al.*, 2013). Consequently, the release of cultivars with improved resistance to drought conditions and with high yield stability is what will drive food security in the twenty-first century (Reynolds et al., 2011; Chapman *et al.*, 2012; Silvas *et al.*, 2015; Nithya *et al.*, 2020).

Rice, being a drought susceptible crop with semi-aquatic behavior, shows damage to physiological, morphological and molecular features that account for growth and development under water stress conditions. The rice grain yield under water stress prone condi-

tions can be maintained with the help of various strategies like, exploitation of diversified germplasm (Beena *et al.*, 2017); effective management practices (Beena *et al.*, 2013) and by utilizing the endophytes related to rice (Johnson *et al.*, 2014).

PLANT PHENOTYPING

Although the concepts were developed by Gregor Mendel, the terms 'gene', 'genotype', and 'phenotype' were only introduced later by the Danish botanist Wilhelm Johannsen in 1909. The terminology regarding phenotyping is not completely clear-cut and also the terms 'phenotype' and 'phenotyping' are interpreted in diverse ways (Mahner and Kary, 1997). The potentiality of mankind to pick the most efficient performing individuals of plant species for domestication – and thereby to 'phenotype' – has been one among the prerequisites for the evolution of human civilization.

'Phenotype' refers to a group of traits that is distinguishable by direct inspection or by some finer methods or through an outline that links interactions between the genotypes and the environment (Johannsen, 1911; Walter *et al.*, 2015). 'Plant phenotyping' necessitates the utilization of advanced devices and methods for quantitative analysis of phenotypes and their description to comprehend the multiplex interplay between genomics and phenomics at distinct levels of integration, e.g., from subcellular, cellular, tissue, or even chloroplast to the whole plant level (Houle *et al.*, 2010; Granier and Vile 2014). Earlier phenotype of a plant was measured by manual methods, e.g., a ruler, a weighing balance, and other means available at that time (Fahlgren *et al.*, 2015), but nowadays plant phenomics make use of a number of non-invasive sensors for analyzing phenotype of vast populations which aims to speed-up identification of plants with high stress tolerance, and to come up with high yielding genotypes, which is anticipated to assist us in achieving overall goal of high sustainability in agriculture (Furbank and Tester, 2011; Fiorani and Schurr, 2013). Incorporation of optimized phenotyping processes to achieve efficient and maximally controlled outcomes lead to high throughput screening of plants. Crop production for research purposes is a complex process involving experimental designs, growth techniques and management practices to data acquisition and strategy analysis. The phenotyping strategies thus pose a myriad of questions and decisions related to its accuracy, precision, automation and adaptation as the efficient synthesis of 21st century crops will be increasingly dependent on using that knowledge (Cobb *et al.*, 2013).

Adequacy for precise phenotyping under authentic conditions probably constitutes the foremost limiting factor for the advancement of genomic studies on drought tolerance. To attain an accurate phenotyping, it is also important to manage the stress levels, time intervals and regular examination. The intensity of drought stress often varies from year to year and within fields resulting from variations in soil composition which determine the capability of the soil to retain water. Often field studies designed to gauge genetic diversification in drought tolerance are faced with disparate stipulations. There is a need for high precision because the distinction may be slight and subtle, and comprehensive physiological analysis (i.e., photosynthetic activity assessment) are strenuous when an extensive number of genotypes are involved.

TECHNOLOGICAL ADVANCEMENT IN PHENOTYPING

Phenotyping and genotyping are major components of analytical breeding. Advances in genotyping technologies had made it quicker, -

while phenotyping had lagged behind and still seems to be a rate-limiting step (Kumar *et. al.*, 2016). Recently, image-based sensors have emerged as a *de-novo* technique of accurately phenotyping large sets of genotypes in a high throughput manner. Non-invasive sensors and advanced computational platforms aid in drawing out the plant attributes. Plant phenotyping is the comprehensive assessment to analyze plant traits related to growth and yield with accuracy and precision. Major plant traits are related to root morphology, biomass, yield-related traits and abiotic stress response. Nowadays, high throughput phenotyping platforms have been used in controlled environment conditions. Under controlled conditions, the environmental effect is also nullified to a great extent so that the real genetic potential of genotype is expressed in terms of phenotypes. The novel phenotyping platforms entails: Non-invasive sensors, Automated data processing to procure phenotypic traits of significance, Mechanized delivery of sensors to plants or vice-versa, Robotized plant culturing and mechanized analysis of processed data in a data management channel.

Non-invasive sensors commonly employed in the automated plant phenotyping hinge on spectrometry (IR Thermometry, FTIR, NIR meter, Hyperspectral radiometers, etc.) and spectroscopy (Visual imaging, Hyperspectral imaging, IR thermography, NIR image analysis, Chlorophyll fluorescence imaging, bioluminescence imaging, fluorescence imaging etc.). Along with the physical state of plants (i.e., growth), advanced sensors monitor their functional, molecular and biophysical processes decidedly as well, as they alter in response to genetic mutation or environmental factors (Houle, 2010).

Most automated phenomics facilities established in the world largely rely on spectroscopy-based image acquisition and analysis capabilities to procure physiological and morphological phenotypes of plants. Technologies that enable this include infrared cameras to scan temperature profiles, spectroscopes to quantify photosynthetic rate, lidar to work out the growth rates and Magnetic Resonance Imaging (MRI) to reveal root physiology. In an approach akin to high throughput DNA sequencing, institutes around the world are building facilities with instruments which can analyse thousands of plants a day.

In general, phenotyping can be assorted into two types: one related to the shoots (above ground) and the other to the roots (below ground). Walter *et al.* (2015) based on the quality of sensors and their performance, structured phenotyping into four classes: (a) RGB (red–green–blue) imaging for measuring size, morphology, growth of plants, architecture of the canopies; (b) thermal imaging of plants or canopy to phenotype temperature and other derived indicators (stomatal transpiration or water status); (c) spectral reflectance/fluorescence of leaves, plants or canopies to analyze the pigments and the biophysical and biochemical processes; and (d) root phenotyping for understanding the physiology and architecture of the root system.

PHENOTYPING ABOVE THE GROUND

RGB IMAGING

Visual digital images help imitate human acuity and furnish the information in digital form which can be seen but expression in data form becomes difficult. The oldest and one of the most important techniques in plant phenotyping is the digital imaging within the visible spectral region (400–700 nm), called red–green–blue (RGB) imaging. RGB images can

be used to measure dynamic aspects of morphology, architecture and growth rate. In in-vitro culturing of plants for acquiring micro-propagation analysis and for measuring growth and analysis RGB imaging had been extensively used. (Smith and Spomer, 1987; Smith *et al.* 1989) in addition to investigating movement and elongation of roots and shoots (Care *et al.*, 1998).

In the plant phenomics facility, plants are grown in greenhouses, on conveyors having radio frequency identifiable (RFID) cars. For recording data, pots are called in the imaging area. The RGB chamber consists of LED tube lights, which activate almost 10 minutes before the image recording so that the light gets saturated within the chamber. According to the plant stage, the height of the imaging platform's lifting and turning device is set up to get the total view of the pot from side view and top view. Generally, images are taken from three angles i.e., 0, 120 and 240 degrees along with the top view. These images are saved in the database.

Once acquired, images are stored in a database. These images are then analyzed using image analysis software like ENVI, Image J, Matlab and Lemna Grid which are used to extract the features of interest. After processing the image, plant traits information is classified in the form of total plant area, plant height, convex hull, caliper length, plant height and different color.

This information is used to calculate growth over a period of plant development. Besides this, abiotic stress effects can be explained based on the leaf senescence, by separating the yellow and green areas of the leaf. These elements can be measured expeditiously and accurately, consequently they can be measured in large populations and mapping populations.. Rajendran *et al.*, (2009) reported that when plants were larger than 100 cm,2, imaging analyses became less reliable indicators of leaf areas. Technological advancement has refined spatial and temporal resolution of the images with unparalleled precision, and enhanced throughput is indeed quite good for statistics, but there is an immense challenge for image comparison, characterization and analysis of large datasets. Furthermore, new devices and procedures are being developed for integrating the underlying genetic and molecular information with processes directing plant -

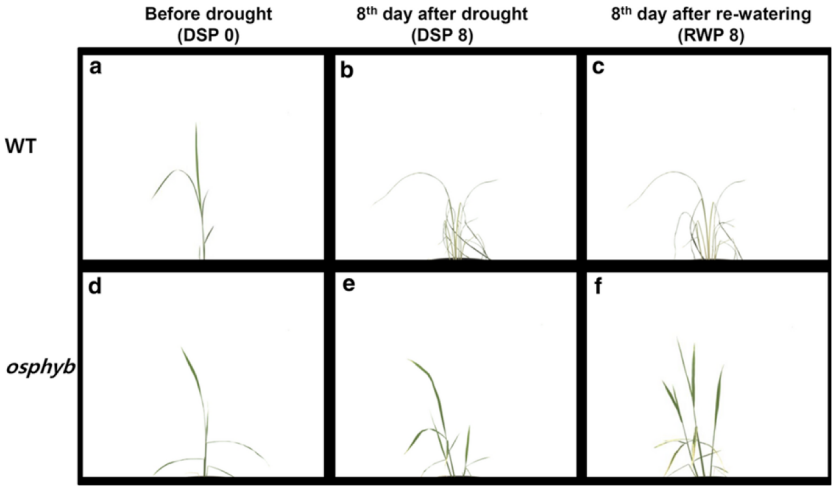

Figure 1. RGB images of Wild Type and *osphyb* mutant plants obtained using image scanners before and after the induction of drought stress and re-watering. (a) and (d) images before drought stress induction; (b) and (e) show images obtained in drought stress phase (DSP) 8 (8th day after drought stress); (c) and (f) are images from re-watering phase (RWP) 8 (8th day after re-watering). (a–c) shows WT images and (d–f) osphyb images (Source: Kim *et al.*, 2020)

growth, development, and physiology (Hartmann *et al.*, 2011; Sozzani *et al.*, 2014; Rousseau *et al.* 2015).

INFRARED THERMAL IMAGING

A thermal image or a thermograph is an image captured in the infrared (750–1300 nm) region of electromagnetic spectrum, and is a firmly established technique for non-invasive measurement of canopy temperature (Jones *et al.* 2009; Berger *et al.* 2010). Infra-Red thermography is a segment of the EM spectrum which emits a particular amount of radiation as a function of their temperatures. High-throughput phenotyping through an infra-red thermography (heat sensitive sensor) relies on the heat produced in stressed plant (Munns *et al.* 2010). Through a combination of careful image capture, image analysis and color classification using IR, it is possible to quantitatively follow the advancement of stressed plants over time. In gauging the stressed plants, IR thermal sensing can furnish expeditious, extensive and effective phenotyping.

When plants exhibit elevated temperature they generally have less water and more infra-red radiation is emitted. A special camera with IR imaging can detect this radiation quite similar to the way an ordinary camera detects visible light. It performs well even in total darkness as the ambient light does not contribute to the imaging. Infra-red thermal images usually have a single-color channel since the camera commonly uses an image sensor that does not differentiate wavelengths of infra-red radiation. IR cameras sense early heat generation in stressed plant or plant organs, mainly in leaves. It was proved that warm parts of the stress tolerant plant indicate high heat compared to cool parts. Detection and discerning of leaf water status reflected in heat generation by IR camera using software can be done under controlled environment. An expression of pattern of colour and average plant / leaf temperature results due to stress induced heat generation. Stress sensitive wild-type plants showed less blue appearance compared to stress tolerant transgenic plants. High-throughput phenotyping techniques using IR thermography saves time for screening of stress tolerance, provide better coverage and is non-destructive in nature.

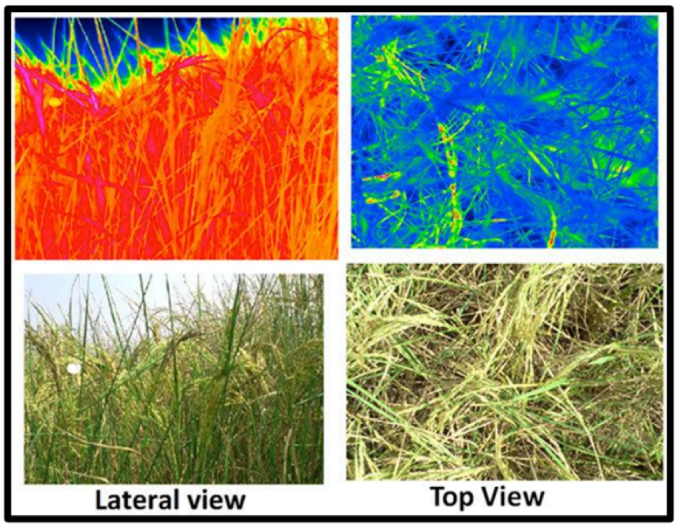

Figure 2. Representative thermal and corresponding visible image of the rice crop canopy captured for experiment (Source: Krishna *et. al.*, 2019)

The opening and closing of stomatal apertures, which regulates the leaf temperatures, provide a link between the thermal images and transpiration rates and responses of stomata (Blum *et al.*, 1982; Hashimoto *et al.*, 1984). Nonetheless, precise monitoring of temperature is a challenge since several factors, e.g., incident radiation, vapor pressure deficit, wind speed, soil moisture and microclimate around the canopy affect leaf temperature and make it strenuous to quantify it under field situations (Walter *et al.*, 2015). In spite of these limitations, this method is indicated to be well suited for phenotyping differential behavior of stomata in grapevine and rice (Jones *et al.*, 2009), for screening of mutants (Wang *et al.*, 2016), and, for observing the impact of differential relative water content succeeding

drought stress in natural accessions of *Arabidopsis thaliana* (Klem *et al.*, 2016).

Kwon *et al.*, (2015) explored the role of IR imaging in the response to stress in a drought tolerant transgenic line of rice expressing CaMsrB2, pepper methionine sulfoxide reductase B2, gene. Stomatal density, stomatal size, net CO2 uptake, and transpiration rate were reported to be higher in Wild type rice plants as compared to drought-tolerant mutant Osphyb (Kim *et al.*, 2020). A likely explanation for the temperature differences suggested by IR images is put forward based on the structure of stomatal pore and transpiration rate. A higher stomatal density and size may lead to an increase in transpiration and a higher rate of water loss under drought stress. There is a correlation of leaf stomatal density with stomatal conductance, net CO2 assimilation, and Water Use Efficiency. Furthermore, the leaf water potential increases with increasing stomatal size (Xu and Zhou, 2008).

IR IMAGES FOR PHENOTYPING OF DROUGHT TOLERANT TRANSGENIC RICE

The complexity of environments and multiple traits render problems to the bioassay of transgenic plants against drought stress in the field conditions. IR imaging is an effective tool to identify drought tolerant genotypes in terms of rapid and comprehensive coverage simultaneously. IR thermography works primarily on heat production and is measured as temperature difference among the tested plants. IR thermography of plants is often linked to some of the physiological characteristics with stress tolerance. For example, in a study of CaMsrB2 transgenic rice plants, thermal images of drought-tolerant transgenic genotypes and their wild types revealed considerable contrast in their thermal images. It was observed that as compared to that of their wild-type plants, the drought tolerant gene induced transgenic lines had lower plant and leaf temperatures.

Siddiqui *et al.*, (2014a) observed that in drought stress, CaMsrB2 expressing transgenic rice genotypes varied from its wild-type genotypes in average plant temperature. The stress-induced changes of plant temperature manifested a significant relationship with the physiological traits of plants such as osmotic potential, stomatal conductance and Relative Water Content.

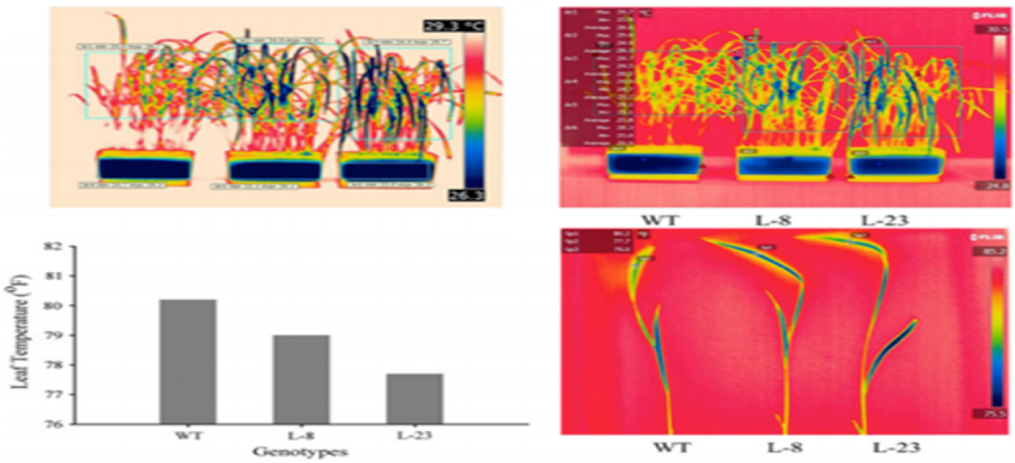

Figure 3. High-tech IR images of CaMsrB2 transgenic plants in drought stress environments. WT wild type, L-8 line 8 carrying single-copy T-DNA insertion, L-23 line 23 carrying two-copy T-DNA insertions. Vertical lines on the bar graph show mean active upper leaf temperature. Images were taken by FLIR SC-620 series camera (Source: Siddiqui *et al.*, 2014)

There are several studies regarding the consistency of IR thermography under drought stress with stomatal conductance and relative water content. Forward-looking IR thermal images of stressed plants revealed substantial variations in leaf temperatures of both transgenic lines as compared to the wild type rice under the drought stress (Siddiqui *et al.*, 2014b). Plant leaf temperature is an instantaneous indicator of internal water status in a drought environment. It was reported by various scientists that immediate indication for responses of plant against drought was captured by IR thermal sensing of drought-imposed plants (Munns, 2002; Sirault *et al*. 2009). Consequently, phenotyping using an IR thermography recognizes drought-tolerant plant attributes with higher efficiency. In drought-sensitive genotypes, leaf and plant body temperatures are increased in water-deficit environments. While in drought-tolerant plants, stomata play a critical role to maintain enough moisture inside the leaf and lower temperatures under water deficit condition (Munns, 2002; Jones *et al.*, 2009), as was seen in the case of CaMsrB2 transgenic lines.

THERMAL IMAGING ESTIMATION OF CANOPY TEMPERATURE DEPRESSION

Canopy temperature depression as estimated by thermal imaging is the variation in temperature of the canopy surface with the surrounding air. CTD is a highly consolidating characteristic resulting from the effects of several biochemical and morphophysiological properties undergoing at the root, stomata, leaf, and canopy levels. In field conditions, to avoid excessive dehydration genotypes with cooler canopy temperature under drought stress, or higher CTD, plants use more of the available water within the soil (Reynolds *et al.*, 2009). IR thermometry can report even delicate differences in leaf temperature in both field and controlled environments. Significantly, data collection - is swift and non-destructive. Plant water balance is a direct measure of drought response in crop plants (Chaerle *et al.*, 2007). This is often associated with plant transpiration process which can explain the variation in leaf temperature and is directly connected to stomatal conductance and leaf temperature. Therefore, CTD has been utilized in breeding programs to screen out stress tolerant or susceptible genotypes particularly under drought conditions. This is on account of cooler canopy temperature which will direct to drought avoidance. Cooler canopy temperature is involved in up to 60 percent yield variation. The main processes linked with drought adaptation are augmented root dry weight, transpiration rate and decreased Canopy temperature during grain formation (Osmont *et al.*, 2007). It is discerned that if water is available, transpiration through stomata results in cooler leaves. When water is limited for transpiration, stomatal closure occurs which raises canopy temperature. This tempering of stomatal conductance as well as leaf transpiration in response to water stress can be detected through thermal imaging and holds the potential for selecting large numbers of plants for CO2 availability and water uptake capacity. Stomatal pores are responsible for cooling the leaf surfaces by facilitating evapo-transpiration and gaseous exchange. While, contrary to this, stomatal closure and reduced transpiration increases leaf or canopy temperature (Deery *et al.*, 2016). Cooler CT is associated with higher grain yield because of more stomatal opening, exchange of gases and maximum photosynthetic rates. Cooler CT is in addition linked with deeper roots, and higher grain yield (Pinter *et al.*, 1990).

IMAGING OF SPECTRAL REFLECTANCE

A large segment of sunlight falling on the plant surface is reflected; however, pigments in plant leaves absorb most of the visible light, except, of

of course, some green light; this is mostly used for photosynthesis. However, a small fraction (3–6%) is dissipated as heat and as fluorescence. The reflected signal provides information on the absorption properties of pigments present in plant leaves; this signal has been used in remote monitoring of various biophysical phenomena for the last several decades (Malenovsky´ *et al.*, 2009). Imaging spectroscopy uses multispectral or hyperspectral sensors for recording reflectance signals resulting from complex photon-vegetation interactions; multispectral sensors measure reflectance at selected discrete bands, whereas hyperspectral sensors measure reflectance in the range of 400-700 nm with maximum upto 2500 nm.

Using principal component analysis and band-band correlation methods significant wavelengths with no reference to leaf biochemical properties (Song *et al.*, 2011) concluded that the narrow bands based on hyperspectral reflectance data present great potential for discriminating rice of varying growth conditions and for screening stress in rice vegetation. These selected wavelengths manifest substantial potential use for the designing of future sensors.

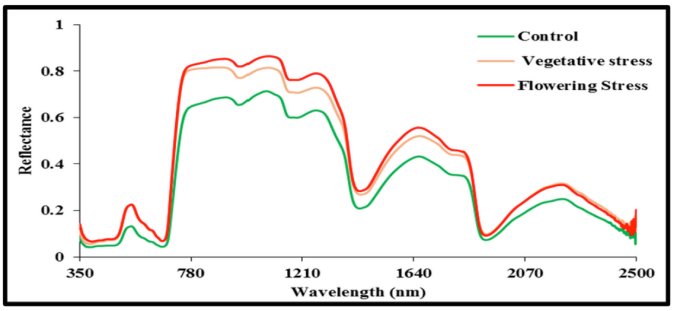

Figure 4. Variations in spectral signatures of rice leaves at different water-deficit stress levels in visible, near infrared (VNIR) and shortwave infrared (SWIR) regions (Source: Das *et. al*. 2018)

IMAGING OF CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence refers to the re-emission of the light energy which had not been utilized for photochemical reactions. Heat loss occurs when excitation energy within pigments is lost as heat; often termed non-radioactive decay or thermal deactivation. Fluorescence occurs mostly from chlorophyll a of PSII in the red region of the spectrum (685 nm) and therefore it is emitted as red light. More than 90 percent of absorbed light is utilized by photosynthesis. Only about 1 to 2 percent light is utilized by the fluorescence process. (Maxwell and Johnson, 2000). Light absorbed by chlorophyll molecules energizes valence electrons of chlorophyll to an excited state. These excited electrons return rapidly to their ground level and release the absorbed energy. Utilization of this energy in the process of photosynthetic photochemistry is most important. The other processes are fluorescence, heat loss, excitation energy transfer to non-fluorescent pigments and quenching by quencher. Chlorophyll fluorescence is an indicator for efficiency of photosynthesis by utilizing detailed information about photosystem II. It reflects the influence of maturation, senescence, drought, heat, cold stress factors on plants. Chlorophyll fluorescence provides a non-destructive tool to investigate these effects on different crops.

Chlorophyll fluorescence (ChlF) is only 2–4% of the reflected irradiance, but it is highly informative and has been successfully used in both basic as well as in applied research for determining photosynthetic efficiency and other photochemical as well as non-photochemical activities. This is of particular importance since fluorescence changes during both biotic and abiotic stress in vivo (Kalaji *et al.*, 2014.; Ruban, 2016).

In addition to chlorophyll a, several other components also fluoresce; these include ferulic

acids, some phenolics, NADP(H) and flavonoids; which are largely located in the upper epidermal part of plant leaves (Morales *et al.*, 1996). The members of this group emit fluorescence in the blue–green spectral region maxima *450 nm (blue band) with a shoulder *520–530 nm (green band) (Cerovic *et al.*, 1999). The features of the fluorescence bands (i.e., intensity, peak position, area under the spectrum) and their ratios are often used as stress indicators in plants (Malenovsky´ *et al.*, 2009).

strongly depends on the root system architecture (RSA) and its function. However, inclusion of RSA traits into breeding programs has been hampered because of lack of high-throughput tools for its characterization under field conditions (Zhu *et al.*, 2011). Based on laboratory or field conditions, different techniques are employed for root phenotyping.

Initially, digital cameras and scanners were used to record 2D images of the root system followed

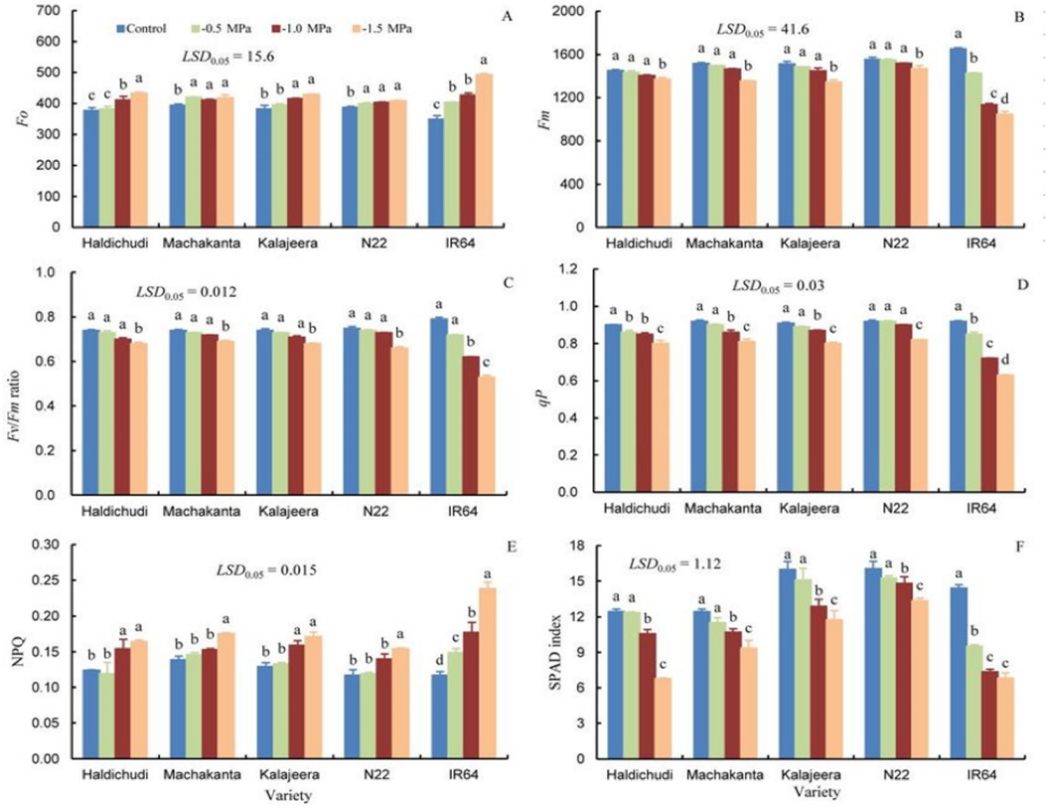

Figure 5. Minimum fluorescence (Fo), maximum fluorescence (Fm), photochemical efficiency of PSII (Fv/Fm), photochemical quenching (qP), non-photochemical quenching (NPQ) and soil and plant analyzer development (SPAD) chlorophyll index in leaves of 5 rice varieties grown in different concentrations of polyethylene glycol (PEG) (Source: Mishra and Panda, 2017)

ROOT PHENOTYPING

Roots are the basic anchorage and absorption organs of the plant and they are imperative for studying the plant's response during varied developmental stages and during stressful environments. Understanding the physiology of the root system is as important as that of the shoots since the performance of all plants -

by their analysis via imaging softwares. For practical reasons, plants were grown either hydroponically or on gel/agar-based growth systems for 2D imaging. Recently, Rattanapichai and Klem (2016) had developed a new root phenotyping system, in which roots were grown on black filter papers with a re-circulating micro-irrigation system between two black plastic foils. This system has been particularly -

utilized to study nutrient deficiency in barley but it holds a promising future for screening root system architecture and its changes during the development of roots in lab conditions. Further, fluorescence imaging can be used for detailed investigations of functions of various compounds and their roles in the development of roots. Seminal and lateral roots have varying angles of branching, density and length and also the kinematics of individual root growth within the root system. This can be analysed through softwares such as SmartRoot, RootScan, AutoRoot etc. (Lobet *et al.*, 2011). Three-dimensional visualization of roots grown in rhizoton or soil column for growth possible through X-ray based computed tomography is another excellent method (Hargreaves *et al.* 2009). Jahnke *et al.* (2009) have used still other sophisticated methods: magnetic resonance imaging (MRI) and positron emission tomography (PET) and their combination for clear and accurate 3D phenotyping of RSA.

Traditionally, the field methods employ excavation of the soil around the root system for the analysis of RSA; further, improved image analysis had been used for increasing throughput (Zhong *et al.*, 2009), but this is a laborious approach with serious limitations such as lack of information about fine roots and not allowing the same plant to be assessed more than once. To overcome these limitations, transparent tubes called minirhizotrons have been developed that can be installed vertically, horizontally, or at some various angles in the field and many software packages have been developed for further analyses of such data (Zhu *et al.*, 2011). With the growing importance of RSA directly under field conditions, where it is much more relevant, a core-break method, developed by Bohm (1979), is now being frequently used because of its better throughput as compared to labor intensive methods such as Augur sampling, ingrowth cores, pinboards, and trenching (Walter *et al.*, 2015).Earlier this method was based on manually counting the roots at different cores of the soil but now imaging cameras are employed to perform this task. In 2016, Wasson and coworkers used an innovative portable system incorporated with the imaging of blue fluorescence for root phenotyping. Other methods, e.g., ground penetrating radar (GPR) (Zenone *et al.*, 2008) and electrical resistivity imaging (Amato *et al.*, 2009), have also been used for non-invasive imaging of roots in field grown plants and trees.

TECHNIQUES USED FOR ROOT PHENOTYPING

Initially root phenotyping was developed in the laboratory followed by checking its applicability at the field level. Some level of automation is incorporated with imaging and processing of

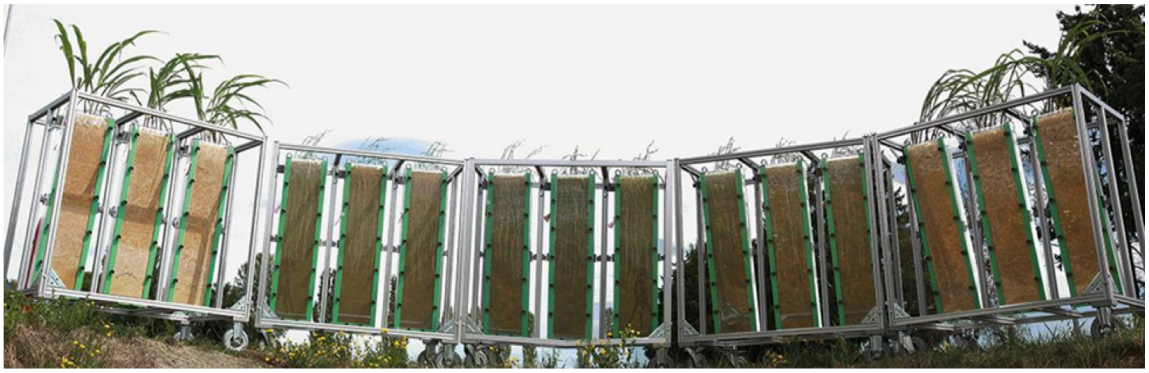

Figure 6. Root assessment along a transparent wall or within a soil column is performed by using X-ray–based computed tomography (CT) to visualize 3-D root configuration (Source: Grift *et. al*, 2011).

images in root phenotyping techniques. Root phenotyping via imaging had accelerated the process with the use of several available softwares such as EZ-Phizo, Smart Root, ImageJ, Root Nav, IJ_Rhizo, Root System Analyzer and Root Trace. Commonly-used systems for root observation are based on soil-less growth media. For this purpose, different techniques are used to grow plants, e.g., growing plants in paper rolls, gels in air regularly sprayed with nutrient solution or in aerated aqueous solutions.For enhanced clarity, plants are grown hydroponically in transparent plexiglass nail board sandwiches which provide mechanical resistance. For better nutrient circulation inside these sandwiches, they are filled with glass beads of 1.5 mm size. The measurement is done for various root traits like total root length, root branching angles etc., though visual rating or imaging. High resolution cameras and scanners are employed for segregating and resolving lateral roots from main roots by using the individual root diameter as the selection criteria to differentiate both kinds of roots via WinRhizo software. RSA can be analyzed through Smart Root software for the measurement of growth kinematics and branching angles of individual roots of a root system. These systems require some manual input for such analyses (Nagel *et. al.*, 2009).

Soil, an opaque mixture of minerals, water, air, organic matter and countless organisms, proves to be a taxing factor for image processing when used as the growth medium. For a near to natural soil medium, soil filled rhizotrons/columns are being used to study soil compaction of drying effects as it is difficult to do such studies in a totally artificial medium.Hence to perform such studies, soil or any other growth substrate is filled inside the columns or Rhizotrons. Then root assessment along a transparent wall or within a soil column is performed by using X-ray–based computed tomography (CT) to visualize 3-D root configuration (Grift *et. al*, 2011).

Due to inherent complexities in the root system architecture, it poses certain limitations for the effective application of current root phenotyping approaches under field conditions for assessing the developed root system in marker assisted selection. (Perkons *et. al.*, 2014; Beena *et al.*, 2018). It is very difficult to assess roots optically in the field, unless one needs to dig them out or approach them by making a tunnel.

In field research studies Mini-rhizotron systems are used extensively. It consists of Plexiglass tubes to which a small camera or scanner is -

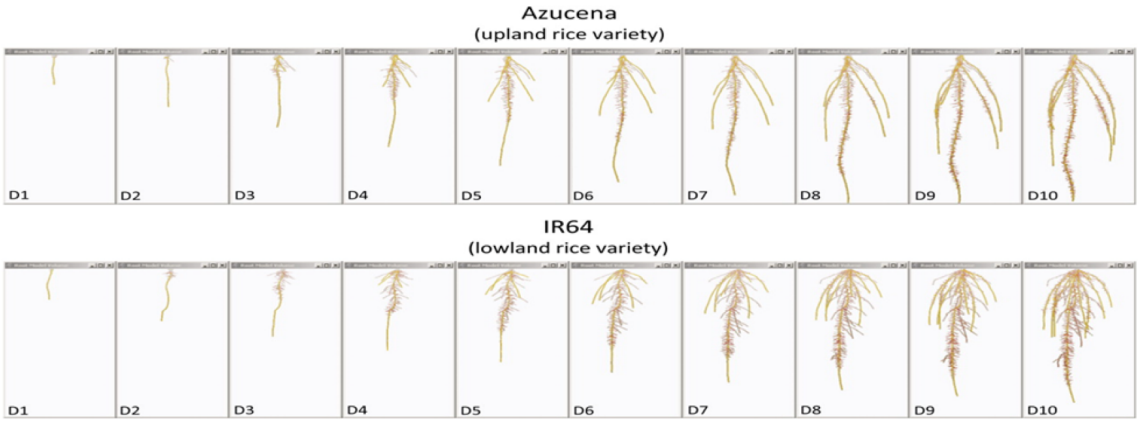

Figure 6. The 3-D root system models generated from daily imaging of root systems over a 10-d period using the RootReader3D software (day 1 [D1]–day 10 [D10]). The primary and crown roots were selected and labeled, allowing for dynamic tracking of root type-specific growth features (Source: Clark *et al.*, 2011).

attached. It is thrust into the soil to analyze the root soil surroundings. Through minirhizotrons, limited genotypes may be monitored. Various indirect approaches for studying RSA are also available such as root pulling resistance or analysis of ABA content in the leaf.

Techniques like X-ray computed tomography (CT) allows us to visualize the root system architecture (RSA) underneath the soil, non-destructively and in a three-dimensional (3-D) format. As there is very little soil disturbance X-ray CT is a very good tool for root phenotyping.In this method, roots are excavated first from the soil and then washed, imaged and finally analyzed with commercially available softwares (Perkons, 2014). CT has various advantages over other destructive methods. Though other different non-invasive 3-D visualization procedures exist, X-ray computed tomography (CT) is considered a good technique for soil-root interaction studies.

SOME AVANT-GARDE OF PLANT PHENOTYPING

The introduction of new and experimental ideas and methods in plant phenotyping using artificial intelligence is on the cusp of an explosion of inventions and innovations. In many institutes across the world high-throughput phenotyping platforms using fully automated, robotized and computer-controlled sensors and data generation systems are utilized. The various metabolic, biochemical and signaling pathways are better understood using these assorted systems and can be used for an insight into plant performances. This will potentially speed up the progress of research in the field of plant physiology and breeding. (Mishra *et al.*, 2016).

The contemporary plant phenotyping systems are of varying dimensions, where many plants can be grown in a controlled environmental system with fully automated light and mechanized irrigation facilities. Usually, each plant grown is exposed to a fixed camera unit which has sensors either by a conveyor belt where the camera unit is fixed or by a robotic camera where the plants are kept in a place for measuring the required phenotypic characteristics. Cruz *et al.*, (2016) have demonstrated an innovative phenotyping system, DEPI (dynamic environmental photosynthesis imager), a high throughput phenotyping platform that comprehensively reveals the mechanisms and genetic foundation of photosynthetic responses that appear due to dynamic or fluctuating environmental conditions. Since there is no need for moving the plants or sensors a large number of plants can be measured simultaneously.

Dhondt *et al.*, 2013 observed that the whole plant phenotyping requires advances in automation which will significantly increase the throughput by enhancing the screening capacity in controlled as well as field conditions. For phenotyping under field conditions, customized sensors can be fitted on robots, in unmanned aerial systems e.g., in drones, or in airplanes (Haghighattalab *et al.*, 2016). A study by Kawamura *et al.*, (2020) investigated the potential of UAV systems on field-based phenotyping and focused especially on the plant height as a case study for upland rice field in Laos and they concluded that enhanced accuracy can be obtained with optimal higher resolution images from lower altitudes with ample camera settings, although an interplay happens between efficiency and accuracy. In comparison with images from robots, the sensors on UAVs give a distinctive outlook of the plant growth. The maps from the drone supply indications on the time and yield of heading, images using robots could be used to observe close up features of the plants. (Burud *et al.*, 2017).

CONCLUSION

The step one regarding the practical development of any stress-tolerant crop plant is to acknowledge the fundamental mechanism of stress tolerance and how it works, which are mostly based on the biochemical and physiological traits. So far there have been continuous studies to explore the application of conventional plant breeding and modern biotechnologies on drought and salinity stress. Exploiting phenotypes to its full potential can lead to an evergreen revolution. However, this has to be done by understanding the underlying mechanisms of root and shoot development, their physiology and also various functions. The current technology, even though rather advanced than what was ten years ago, is still not equipped enough to maneuver the characterization of whole plant phenomics. However, it is imperative to realize that extensive research is required in this area to accelerate the ultimate goal of increasing agricultural productivity.

Plant phenotyping is emerging as a field with lots of opportunities and interests. It entails experiments and measurements of various plant traits, integrated data generation, analysis, management, and integration across numerous platforms. This enables connecting phenotypic and genetic approaches, to come up with climate smart crops by using modern perspectives of scientific knowledge of plants' interaction with its ever-changing environment. There is still a way to go in this field as the sheer volume of data generated through new technologies need to be integrated into research and academics. Crops which can tolerate the ill-effects of climate change, namely drought, can be developed and released then only. Food security and poverty eradication and ultimately the lives of millions of people depend on this. There are challenges ahead concerning the - technological acquisitions of plant phenotyping but the silver linings of a great many opportunities are hard to ignore.

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