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REVIEW ARTICLE

Plant phenotyping: a perspective

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Abstract Sustainable agriculture for feeding increasing population is a foremost global challenge. The "green revolution" based crop productivity has done wonders in the past, but it has limits, and, thus, we are compelled to look for new avenues to increase productivity of important crops. *Plant phenomics* is emerging as a promising area in which many imaging sensors developed in the past are being tested for mapping of genetic information expressed within plant phenotypes, and the integrated use of these sensors may help speed-up unraveling of underlying molecular, biochemical and physiological mechanisms. We provide here a review of methods used for phenotyping and understanding of abiotic stress (drought/cold) tolerance mechanisms in the context of dynamic challenges faced by plants during their life.

Keywords Chlorophyll *a* fluorescence (ChlF) · Cold acclimation (CA) · Drought acclimation (DA) · Hyperspectral imaging · Infrared thermal imaging

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Introduction

Increasing food demand with "shrinking" land area under global warming scenario while maintaining environmental sustainability is a global, social and economic challenge for feeding the increasing human population (Lobell et al. 2011; Thomson 2002; Boyer 2010; Campbell 2013). During the 1970s many developing countries, including India, achieved "green revolution" that has driven a remarkable increase in food productivity with efficient use of improved seeds, chemical fertilizers, agrochemicals, and controlled irrigation (Fresco 2015). The *green revolution* has, however, its limitations in improving the yield potential of crops in the risk prone areas facing harsh environmental constraints, e.g., flood, drought, or temperature fluctuations (heat and cold), and there is no remedy in it to deal with the deterioration of soil quality, water quality, and biodiversity caused largely due to uncontrolled use of agrochemicals and fertilizers (Kesavan and Malarvannan 2014). Ray et al. (2013) have shown that it is impossible to achieve the expected global food production to meet the projected required demands because of slowing down of production in major crops across many growing areas. To bridge the gap between the demand and the production, there is a need of investment in harnessing available scientific knowledge and technological breakthroughs and political will for adapting and implementing new policies towards *evergreen revolution* (*green revolution 2.0*; Pingali 2012; Kesavan and Malarvannan 2014). However, addressing issues of sustainable agriculture for increasing crop productivity with the current pace of our needs is going to be the greatest challenge in the near future, and its success will require multidimensional approach that includes development of programs and management strategies to improve land quality, and precision farming practices;

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further, we will require efficient exploitation of genetic resources for the development of high yielding crops, with enhanced stress tolerant plants, and much more, depending, of course, on the needs of different regional and geographic areas.

In general, certain types of stress are being mitigated with the use of water, fertilizers and pesticides; however, for the sustainability of agriculture and farming practices, reckless use of fertilizers, and pesticides, must be reduced and policy driven research initiatives are needed for exploring genetic features of crops, e.g., those having biotic and abiotic stress tolerance, and efficient water and nutrient use (Kesavan and Malarvannan 2014). There is already significant progress at molecular and genetic levels in *Arabidopsis (A.) thaliana*, a model plant, but regulation and co-ordination of complex molecular pathways and mechanisms governing stress perception and transduction are still largely unknown (Somerville and Briscoe 2001; Valliyodan and Nguyen 2006; Koornneef and Meinke 2010; Provart et al. 2016). Exploration of genetic potential for improving plant photosynthesis and developing high yielding varieties will require a thorough understanding of the underlying mechanisms that contribute to tolerance under adverse environmental conditions (see e.g., Kandoi et al. 2016 for Arabidopsis, but, for a special successful story in tobacco, see Kromdijk et al. 2016). In the recent past, many in Europe, USA, Australia and Asia, have launched research initiatives for developing infrastructure to describe plant phenotypes as a collection of traits necessary for high yield under specific or challenging environmental conditions, and a new research area "Plant Phenomics" is rapidly developing. The major goal is to efficiently use available technologies for the selection of plant genes or the germplasm having better resistance and/or survival strategy to challenging environmental conditions; this is expected to be done by linking genetic and phenotypic traits at high-throughput scale (Furbank and Tester 2011; Fiorani and Schurr 2013). On the other hand, ideas of redesigning plant systems, at different scales, for the efficient utilization of photosynthetic efficiency and performance to increase crop yields is also developing in what one may call *Synthetic Biology* (Ort et al. 2015). Apart from genetic adaptation (natural adaptation), plant acclimation, to micro and macro environmental conditions, plays an important role in mitigating stress factors and in diversification of species; thus, a systematic management and strategy is a prerequisite to address the contribution of factors important at regional scale, while selecting tolerant varieties. Moreover, not much attention has yet been paid to understand the impact of natural dynamic behavior of highly important environmental stimuli (e.g., varying light intensities, temperature and humidity) on plant photosynthesis and their stress tolerance capacity; most of the current investigations have been on plants grown in growth chambers, or in greenhouses with well-defined standard growth conditions. However, many investigations have demonstrated that fluctuating light produces strong phenotypes (Kulheim et al. 2002; Rascher and Nedbal 2006; Tikkanen et al. 2012; Cruz et al. 2016); thus, it is highly relevant to initiate investigations to understand how dynamic behavior of individual as well as multiple environmental situations can improve plant yields.

Here, we will briefly review recent advancement in *plant phenomics* for phenotyping shoots and roots; this will be followed by their application to investigate basic plant traits with emphasis on important abiotic stress factors, e.g., drought and cold. We shall then briefly describe the challenges in plant phenomes and how the new technologies can speed-up the selection of stress tolerant varieties having better strategy of survival during mild to moderate stress.

Plant phenomics

In general, "phenotype" refers to a set of traits that is distinguishable by direct inspection or by some finer methods or through a description that links interactions between the genotypes and the environment (Johannsen 1911; Walter et al. 2015). "Plant phenomics" involves the use of advanced tools and methods for quantitative measurements of phenotypes and their description to understand the complex interplay between genomics and phenomics at different 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). In the past, phenotype of a plant was measured by manual methods, e.g., a ruler, a weighing machine, and other available devices (reviewed in Fahlgren et al. 2015), but today *plant phenomics* uses numerous non-invasive sensors for analyzing the interaction of genotype with the environment which is expressed as a phenotype of large populations with an aim to speed-up identification of plants that have high tolerance to biotic/abiotic stress, and to provide high yielding genotypes, which is expected to help us in achieving overall goal of high sustainability in agriculture (Furbank and Tester 2011; Fiorani and Schurr 2013; Granier and Vile 2014).

Advanced sensors (see Table 1) not only monitor physical state of plants (i.e., growth) but also to a great extent their functional, molecular and biophysical processes, as they change in response to genetic mutation or environmental factors (Houle 2010). In general, we can divide phenotyping into two types: one related to the shoots (above ground) and the other to the roots (below ground). However, based on the quality of sensors and their

Table 1 Methods of plant phenotyping currently employed

	Sensors	Phenotype	References
Shoot phenotyping	RGB (red–green–blue) imaging	Growth rate, morphology, structure, chlorophyll content, nitrogen content	Borhan et al. (2004), Granier et al. (2006) and Hartmann et al. (2011)
	Multi- or hyper spectral reflectance imaging	Pigments and their activity, water deficit, nitrogen content, plant biomass, disease incidence	Berger et al. (2010), Seeing et al. (2009), Kim et al. (2011), Svensgaard et al. (2014) and Bauriegel and Herppich (2014)
	Infra-red (thermal) imaging	Stomatal response, water deficit, disease incidence	Munns et al. (2010) and Chaerle et al. (2004)
	Chlorophyll fluorescence	Photochemical and non-photochemical activity; photosynthetic performance in relation to abiotic and biotic stress	Jansen et al.(2009) and Mishra et al. (2012, 2014, 2016)
	3D mapping (e.g., stereo vision, laser scanning, structured light scanning	Plant morphology, 3D structure	Omasa et al. (2007), Paulus et al. (2014) and Bellasio et al. (2012)
Root phenotyping	RGB imaging	Root growth, branching, kinematics of individual roots	Hund et al. (2009)
	Fluorescence spectroscopy (imaging)	Root phenotyping in soil cores	Wasson et al. (2016)
	X-ray tomography	3D analysis of root architecture and its physiology	Hargreaves et al. (2009)
	Magnetic resonance imaging (MRI)	3D analysis of root architecture and its physiology	Nägel et al. (2009) and Jahnke et al. (2009)
	Positron emission tomography (PET)	Root architecture and its physiology	Jahnke et al. (2009)

performance, Walter et al. (2015) structured phenotyping into four classes: (a) RGB (red–green–blue) imaging for measuring size, morphology, architecture or growth of plants or their 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 for investigating their pigments and their biophysical and biochemical processes; and (d) architecture and physiology of the root system.

Phenotyping of plant shoots

RGB imaging

The oldest and one of the most important techniques in plant phenotyping is the digital imaging in the visible spectral region (~400–700 nm), called red–green–blue (RGB) imaging. Figure 1a shows a typical RGB image of well-watered and 10-day drought induced whole plant rosettes of *A. thaliana*; it is obvious that this image can be used to measure dynamic aspects of morphology, architecture and growth rate. Many open source tools for image processing, and its analysis, are freely available in the public domain, e.g., imageJ (https://imagej.nih.gov/ij/), which is not only used for simple image analysis, but can also be extended for use in handling specific problems. This method has indeed been used for measuring growth and development and for obtaining micro-propagation analysis of in vitro cultured plants (Smith and Spomer 1987; Smith et al. 1989) as well as for investigating elongation and movement of both roots and shoots (Care et al. 1998; Nelson and Evans 1986; reviewed in Leister et al. 1999). A combination of digital video and image analysis has been used to quantify plant growth and growth rate in *A. thaliana* (Leister et al. 1999). Currently, images are being captured in large quantities and at high-throughput scale to analyze the morphology and the growth rates of plants; these methods work well for plants with rosettes such as Arabidopsis (Granier et al. 2006; Walter et al. 2007); however, there are limitations and challenges for complex crop plants with 3D growth with multiple shoots, e.g., for wheat, Rajendran et al. (2009) showed that imaging analyses became less reliable indicators of leaf areas when plants were larger than 100 cm^2. Technical advancement has improved spatial and temporal resolution of the images with unprecedented precision, and increased throughput is indeed quite good for statistics, but there is a huge challenge for doing image comparison, characterization and analysis of large datasets. Further, new tools and methods are now being developed for integrating and coupling the underlying genetic and molecular information with processes governing plant growth, development, and physiology (Hartmann et al. 2011; Sozzani et al. 2014; Rousseau et al. 2015).

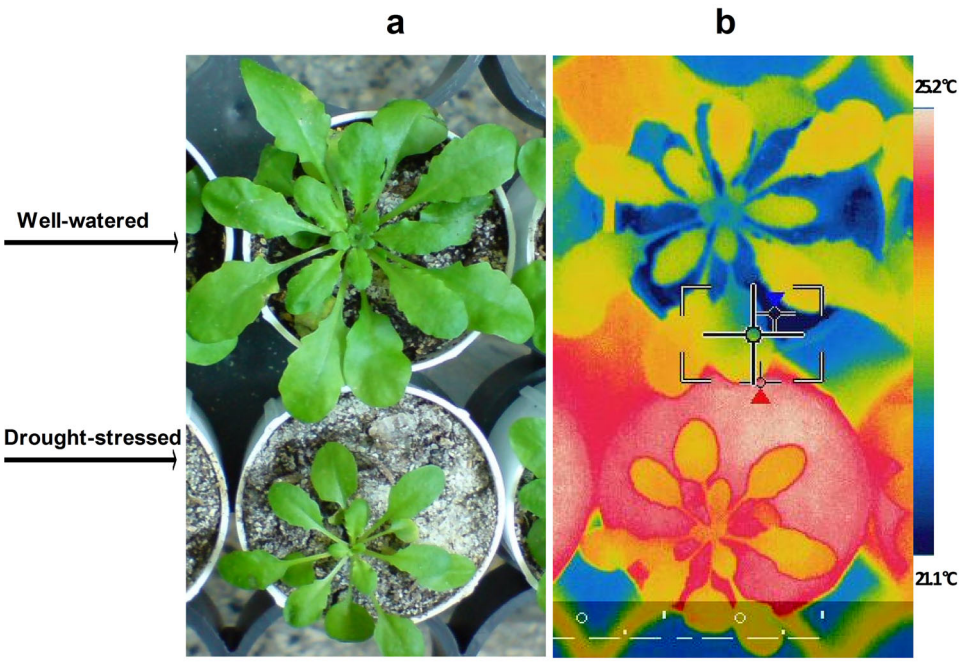

Fig. 1 Digital RGB (*red–green–blue*) image (**a**; *left panel*), and an infra-red thermal image (**b**; *right panel*) of *Arabidopsis thaliana* plants that were well-watered (controls) or water-stressed for 10 days. These photographs can be used to visualize morphology, count the number of leaves, and to calculate the rosette area and the growth rate of plants (Jansen et al. 2009). Further, the thermal image can be used to precisely monitor temperature distribution in these plants (Munns et al. 2010). In this experiment, the scale for temperature ranged from 21.1 (*dark blue*) to 25.2 °C (*white*)

Infrared thermal imaging

A thermal image or a thermograph (Fig. 1b) is an image captured in the infrared (~750–1300 nm) region of electromagnetic spectrum, and this is a well-established method for non-invasive measurement of canopy temperature (Jones et al. 2009; Berger et al. 2010). Figure 1b shows thermal images of rosettes of well-watered and mildly drought-stressed Arabidopsis plants. Drought was induced by withholding water. From the thermal images, we were able to obtain not only the temperature of the soils in the two pots, but also that of the leaves of both well-watered and drought-stressed plants. We know that the opening as well as the closing of stomatal pores regulate leaf temperatures, providing a link between the thermal images and transpiration rates and responses of stomata (Blum et al. 1982; Hashimoto et al. 1984). However, precise monitoring of temperature is a challenge since several factors, e.g., incident radiation, wind speed, vapor pressure deficit, soil moisture and microclimate around the canopy affect leaf temperature and make it difficult to quantitatively measure it under field situations (reviewed in Walter et al. 2015). Despite these limitations, this technique has been demonstrated to be well suited for phenotyping differential behavior of stomata in grapevine and rice (Jones et al. 2009), for monitoring early symptoms of plant diseases (Mahlein et al. 2012), for screening of mutants (Wang et al. 2016), and, for observing the impact of differential relative water content following drought stress in natural accessions of *A. thaliana* (Klem et al. 2016). For recent reviews, see Costa et al. (2013), Walter et al. (2015) and Humplík et al. (2015).

Imaging of spectral reflectance and fluorescence

Reflectance

A large portion of sunlight falling on the plant surface is reflected; however, pigments in plant leaves absorb most of the visible light, except, 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 (reviewed in Malenovský 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 over a wide range of wavelength (e.g., 400–700 nm, or even up to 2500 nm).

Figure 2 shows typical reflectance spectra (450–800 nm) of non-acclimated and cold acclimated *A. thaliana* accession "Tenela" (Te, a native of Finland). Reflectance changes in the visible region are due to high absorption by photosynthetic pigments, while high reflectance in the near-infrared (NIR) region is due to high scattering from the internal leaf tissues (Gates et al. 1965). Cold acclimation modifies not only pigment concentrations, but also metabolic, biochemical and physiological processes, enabling plants to better survive freezing temperatures (Lukas et al. 2013; Mishra et al. 2014); thus,

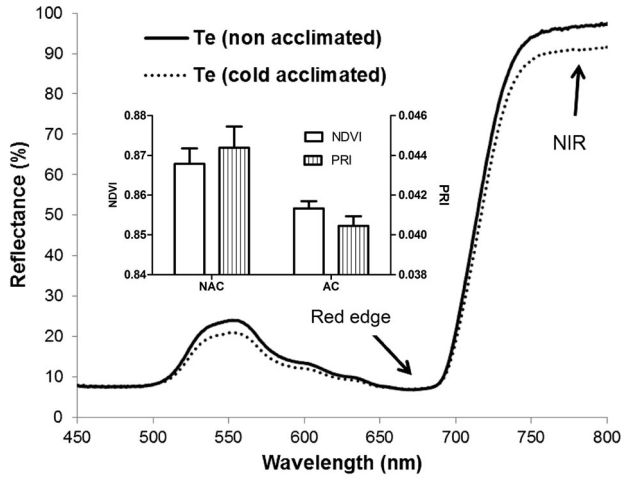

Fig. 2 Reflectance spectra of leaves of non-acclimated [NAC; 6 week old; 22 °C (day)/18 °C (night)] and cold acclimated (CA; 4 °C for 2 weeks) *Arabidopsis thaliana* accession Tenela (Te). These spectra were measured by SM 9000 spectrometer (Photon Systems Instruments, CZ). Cold acclimation induced changes in leaf pigments, and in metabolic, biochemical, and physiological processes are reflected in the reflectance spectra and in the *normalized differential vegetation index* [NDVI = $(R_{NIR} - R_{RED})/(R_{NIR} + R_{RED})$] as well as in the *photochemical reflectance index* [PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$, Gamon et al. 1990]. *NIR* near infra-red

measurements of changes in reflectance spectra of non-acclimated and cold acclimated plants are useful in understanding the physiology behind cold acclimation. In general, significant changes in absorbance occur either due to changes in pigment concentrations or in the underlying physiological processes. As implied above, these can be readily observed in the reflectance spectra, from which reflectance indices (combination of changes at two or more reflectance wavelength) are obtained for quantification of particular pigments or processes (Bilger and Bjorkman 1990; Malenovský et al. 2009). One such example is a measurement at the *red-edge*, the point with maximum slope in leaf reflectance between 680 and 750 nm, where the reflectance changes from very low (because of high absorption by chlorophylls) to very high (high leaf and canopy scattering) value, in combination with reflectance at the near infra-red band (NIR, ~750–1200); the latter, in turn, is calculated from normalized difference vegetation index [NDVI = $(R_{NIR} - R_{RED})/(R_{NIR} - R_{RED})$, where R is the wavelength at the subscripted wavelength region; Rouse et al. 1974]. This index measures what we may call "greenness" (chlorophyll concentration) on land surface; further, this can also be used for the estimation of above-ground biomass, and leaf area index (LAI) (Rhew et al. 2011; reviewed in Malenovský et al. 2009). Gamon et al. (1990) observed dynamic changes in leaf reflectance spectrum at 531 nm, after sudden transition of the leaf from the dark to the light environment. These changes were attributed to light-induced dynamic transformation of violaxanthin to zeaxanthin (a photo-protective pathway), and, this process could be observed remotely by photochemical reflectance index [PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$; Gamon et al. 1990, 1992]. Further, Gitelson et al. (2003, 2006) have developed a model, based on three wavelength dependent indices, and have demonstrated its usefulness in estimating, remotely, amounts of chlorophylls (Chls), carotenoids, and anthocyanins. In the infrared region (~1200–2400 nm), there is low reflectance at certain wavelengths because of absorption by water, proteins, and some carbon-containing compounds, and, this is being successfully utilized in remote sensing (see Curran et al. 1992). *In summary*, several features of reflectance signals can be used in plant phenotyping and many appropriate sensors have already been installed at several phenotyping platforms (for details about phenotyping platforms, see http://www.plant-phenotyping.org/).

Fluorescence

Chlorophyll *a* 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 measuring the efficiency of photosynthesis (especially that of Phtosystem II), as well as some other photochemical and non-photochemical activities. This is of particular importance since fluorescence changes during both biotic and abiotic stress in vivo (for basics and applications, see Govindjee 1995; Baker 2008; Govindjee et al. 1986; Papageorgiou and Govindjee 2004; Kalaji et al. 2014, 2016; Ruban 2016).

In addition to chlorophyll *a*, several other components also fluoresce; these include ferulic acids, some phenolics, NADP(H) and flavonoids; some of these are located in the upper epidermal part of plant leaves (Morales et al. 1996). We note that 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); see Morales et al. (1996), Cerovic et al. (1999). In contrast, and, as is well known, ChlF has a maximum at ~685 nm (red band) with a shoulder at ~730–740 nm (far-red band; see chapters in Papageorgiou and Govindjee 2004). The features of these fluorescence bands (i.e., intensity, peak position, area under the spectrum) and their ratios are often used as stress indicators in plants (Buschmann et al. 2000; Mishra and Gopal 2008; reviewed in Malenovský et al. 2009). For example, the ratio between red and far-red fluorescence (e.g., F685/F735) decreases with increasing chlorophyll concentration because the red band is highly re-absorbed by chlorophylls, while the far-red band is not (Buschmann 2007). The blue–green fluorescence, mentioned above, has been shown to be either constant (static)

or to slowly change with accumulation of the fluorophores, during growth or in response to environmental stress stimuli. Thus, changes in ratios of blue to red (F450/F685) and blue to far-red (F450/F735) fluorescence bands (excitation with UV light) have been related to physiological development of leaves (Stober et al. 1994), and used as markers of stress and nutrition availability (Chappelle et al. 1984).

Chl *a* fluorescence is highly dynamic and provides us important insights on several molecular processes from femtoseconds to minutes (Dau 1994; Govindjee 1995; Baker 2008; Ruban 2016). Semi-high-throughput platforms have already been used to screen ChlF emission from whole rosettes of *A. thaliana* (Jansen et al. 2009; Woo et al. 2008) and of tomato plants (Mishra et al. 2012). Maximum quantum efficiency of PSII photochemistry is often inferred from the ratio of variable to maximum (F_V/F_M) Chl *a* fluorescence (see Govindjee 2004); this was, however, observed to be insensitive in detecting early drought effects (Jansen et al. 2009; Woo et al. 2008). On the other hand, several parameters, such as non-photochemical quenching [$NPQ = \left(F_M - F'_M/F'_M\right)$, where F'_M is the maximum fluorescence in light adapted state] of the excited state of Chl *a*, effective quantum efficiency of PSII ($\Phi_{PSII} = \left(F'_M - F_S\right)/F'_M$), and steady state fluorescence (F_S), have been shown to be more sensitive to changes in physiological state of the plant, such as during mild leaf-water deficit (Mishra et al. 2012).

Figure 3 shows images of ChlF parameters F_V/F_M and NPQ in leaves of nine *A. thaliana* accessions; these images show that the quantum yield of PSII photochemistry is homogenous in all leaves, but there is a high spatial heterogeneity of NPQ across the leaves, with visibly low heat dissipation in the mid and secondary veins (for details, see Nedbal and Whitmarsh 2004). Matouš et al. (2006) used pattern-recognition based statistical methods, in which statistical classifiers and feature selection algorithms (Pudil et al. 1994) were used to exploit heterogeneity among time resolved ChlF image pixels; they searched for combination of images that provided high discrimination between groups to be compared. The obtained combinatorial images were very powerful in predicting detection of biotic stress (Matouš et al. 2006), in discriminating between species of *Lamiaceae* at very early stage of their growth (Mishra et al. 2009), in classifying cold tolerance in *A. thaliana* accessions (Mishra et al. 2011, 2014), and in screening leaf–water-deficit also in *A. thaliana* accessions (Mishra et al. 2016). For the characterization, and use of Chl *a* fluorescence transients, usually 20–30 min of dark-adaptation of photosynthetic samples is required and this is a constraint for high throughput sensing (Rutherford et al. 1984; Groom et al. 1993). Mishra et al. (2016) have recently utilized the advantage of adaptive growth irradiance of plants and demonstrated that ChlF transients, measured in the presence of half of the adaptive growth irradiance (without pre-darkening of samples), preserved several features of ChlF transients and their parameters, and, thus, use of this new protocol can significantly increase throughput capacity of this method. Mishra et al. (2016) have also demonstrated the usefulness of a new protocol for screening drought stress in six natural

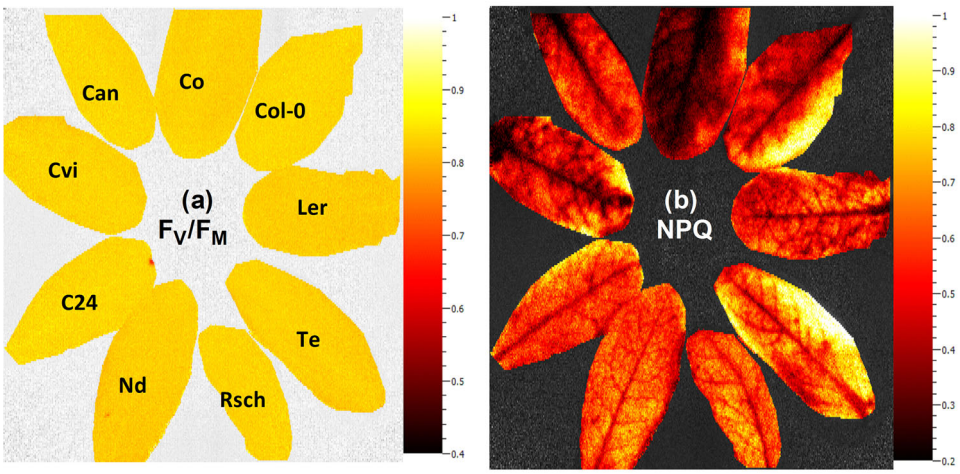

Fig. 3 Images of chlorophyll *a* fluorescence (ChlF) parameters: (**a**; *left panel*) maximum quantum efficiency of PSII photochemistry [F_V/F_M, where F_V is variable fluorescence defined by $F_M - F_O$, F_M = maximum fluorescence of dark-adapted leaves, and F_O = minimum fluorescence when Q_A is fully oxidized], and (**b**; *right panel*) non-photochemical quenching [$NPQ = \left(F_M - F'_M\right)/F'_M$] of ChlF, in leaves of nine different *Arabidopsis thaliana* accessions. These images can be used to monitor spatial distribution of physiological processes, e.g., homogeneous distribution of F_V/F_M, representing quantum yield of PSII; it is uniform over the leaves of all the nine accessions, whereas NPQ is highly heterogeneous across the lamina of the leaves: the mid-veins and the secondary veins are shown to have very low heat dissipation as compared to other part of the leaves

accessions of *A. thaliana* accessions, and have found that in combination with combinatorial imaging, leaf-water deficit (drought) can be "sensed" (detected) at a very early stage of their initiation. For use of fast Chl F transients, the so-called OJIP transient (Stirbet and Govindjee 2011), on phenotyping barley varieties, see Oukarroum and Strasser (2004), and two drought-stressed trees, see Salvatori et al. (2016). Another unexplored area for phenotyping is fluorescence lifetime imaging microscopy. For a study on mutants of single cells of plants, see Holub et al. (2007).

Root phenotyping: from laboratory to field

Understanding the physiology of the root system is as important as that of the shoots since the performance of all plants 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 (for reviews, see Lynch and Walsh 1998; Zhu et al. 2011). Initially, digital cameras and scanners were used to record 2D images of the root system followed by their analysis via imaging softwares. For practical reasons, plants were grown either hydrophonically or on gel/agar based growth systems for 2D imaging (reviewed by Zhu et al. 2011). Recently, Rattanapichai and Klem (2016) have developed a new root phenotyping system, in which roots were grown on black filter papers with re-circulating micro-irrigation system between two black plastic foils (Fig. 4a). Thus far, use of this system has been tested for studying nutrient deficiency in barley, but, in the near future, this system is expected to be used for screening RSA and its dynamics in laboratory settings during root development. Further, fluorescence imaging (Fig. 4b) can be used for detailed investigations of functions of various compounds and their roles in the development of roots. Usually, software such as *SmartRoot* allows analysis of RSA, where seminal and lateral roots have different branching angles, length and density, and there may be different growth kinematics of individual roots within the root system (Lobet et al. 2011). Another powerful method is X-ray based computed tomography (CT), which provides 3D visualization of plant roots grown in a rhizotron or a growth column filled with soil or other growth substrates (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;

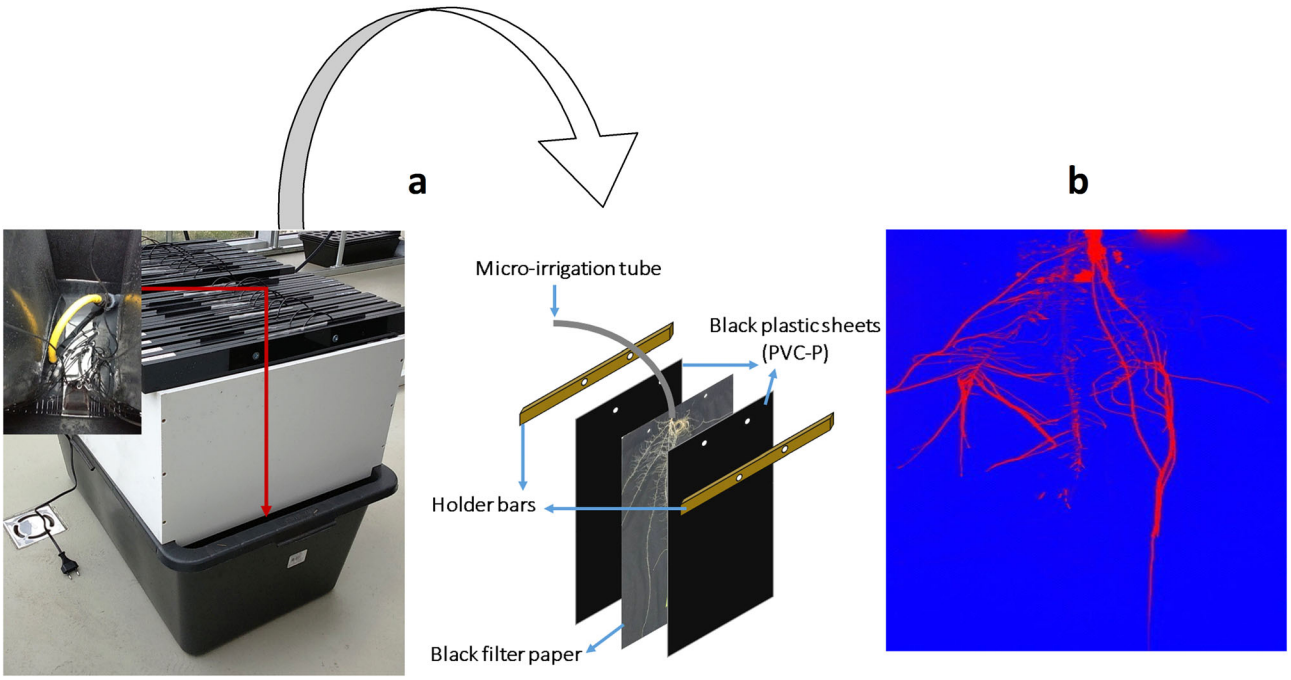

Fig. 4 (**a**, *left panel*) a complex set-up for root phenotyping with growth units [fitted with two plastic holder bars and two sheets of black plastic foils (PVC-P)], and micro-irrigation system inside the reservoir tub (tube fixed between holder bars and black filter paper as a substrate for root growth); (**b**, *right panel*) fluorescence of root system measured by the open version of fluorescence imaging system (Photon Systems Instruments) using UV excitation (λ_{max} = 365 nm); emission was collected using a 530 nm (±10 nm) filter. Figure 4a is from Rattanapichai and Klem (2016), reproduced with permission

further, improved image analysis has been used for increasing throughput (Zhong et al. 2009), but this approach is labor intensive; it may miss information on fine roots, and it does not allow repeated observations on the same plant. To overcome these limitations, transparent tubes called *minirhizotrons* have been developed that can be installed vertically, horizontally, or at a various angles in the field (Bates 1937), and many software packages have been developed for further analyses of such data (reviewed by 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; for a review, see Walter et al. 2015). In this method roots were manually counted at different cores of the soil, but now imaging cameras are being used. Recently, Wasson et al. (2016) have developed a portable system integrated with imaging of blue (peak, ~440 nm) fluorescence (from phenolic and flavonoid compounds) and have used it 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.

Current state-of-the-art of plant phenotyping

We are now confident that the integrated use of currently employed imaging sensors in plant phenotyping has a high potential in speeding-up progress for the better understanding of plant performance and for providing links between the gene function and environmental responses on various signaling, metabolic and biochemical pathways, and processes. Further, many fully automatic, computer controlled, and high-throughput phenotyping platforms are now available at many research institutes across the world (Granier et al. 2006; Walter et al. 2007, 2015; Reuzeau et al. 2005; Hartmann et al. 2011; Granier and Vile 2014; Humplík et al. 2015; Flood et al. 2016; Mishra et al. 2016). Currently employed plant phenotyping systems are of different sizes, where many plants (from hundreds to thousands) can be grown in a growth system with fully automated light and irrigation facility. Usually, each plant in the growth system is accessible to a camera unit (installed with required sensors) either by a conveyor belt (camera unit is fixed) or by a robotic camera (plants are fixed) for measuring relevant phenotypic traits. The camera unit may capture images or sequence of images from different views (e.g., top view, side view); further the unit may be equipped with a turntable where the plant can be lifted or rotated for recording as many images as needed. However, in both conveyer belt and robotic camera phenotyping units, there are certain limitations, e.g., in the former case, size of pots is limited affecting root growth and thus the uptake of nutrients and water, while in the latter, plants are in a more natural state, but the camera is reachable to only limited number of plants. Cruz et al. (2016) have now tested another phenotyping system, DEPI, a dynamic environmental photosynthesis imager, in which a set of five cameras could screen large number of plants in the chamber in which it was possible to provide daily irradiance much closer to that available in *nature*. We note that Cruz et al. (2016) were able to reliably identify phenotypes that were transient and highly dependent on environmental conditions and developmental factors.

The automatic phenotypic platforms have vastly improved the screening capacity and the focus of research at many institutions has already been broadened from single plants under controlled environment to real life applications, i.e., many plants in robust greenhouses and under field conditions (Dhondt et al. 2013; Walter et al. 2015). For field phenotyping, dedicated sensors can be installed in robots, in unmanned aerial systems (UAS), e.g., in drones, or in airplanes (Zhang and Kovacs 2012; Liebisch et al. 2015; Großkinsky et al. 2015; Haghighattalab et al. 2016). This exciting and highly promising field is still in its infancy of its development, and several research groups around the world are now developing relevant protocols, tools and methods, including new softwares for easy handling of massive image datasets, since the readout of large number of image datasets generated from thousands of plants measured at high-throughput screens is indeed a tedious task (Walter et al. 2010; Furbank and Tester 2011; Humplík et al. 2015).

In general, the available techniques for phenotyping of shoots and roots are quite expensive and are going through testing phases, mostly by experts in the field. However, initiatives are required to broaden the application of instrumentation, and communication of data, results and relevant messages from experts to researchers in the developing world, and to the breeders so that they can not only get direct benefit, but also contribute in the collection of high quality data from several regions around the globe. Recently, David Kramer (Michigan State University, USA) and his coworkers have launched an initiative in this direction by providing a new handheld device, MultispeQ (https://photosynq.org/), that phenotypes many photosynthetic parameters, e.g., Φ_{PSII}, NPQ, photosystem II photoinhibition, light-driven proton translocation and thylakoid proton motive force, and regulation of chloroplast ATP synthase (Kuhlgert et al. 2016). The highlight of MultispeQ is that data can be transmitted from desktops, laptops or even mobile phones to the online portals that

provide a platform to collect data from remote areas that may allow understanding of complex processes in plants and their environment from various locations. We have exciting and promising days ahead of us.

Effect of static and dynamic environment in plant research

During the past decade, investigations in plant sciences were either carried out in laboratory settings, or in growth chambers, or in greenhouses and, thus, plants were exposed to well-defined conditions of temperature and light during their life cycle. Controlled experiments on plants have been highly reproducible; they have contributed immensely to our understanding of basic structural, molecular, biochemical and biophysical processes, as well as of the complex responses to environmental stresses. However, under natural outdoor field conditions, plants are exposed to a dynamic but unpredictable environment where light intensity, temperature and humidity are highly variable according to the time of day, seasons, geography, climate, and the position of leaf within the canopy and that of the cell within the leaf (Murchie and Niyogi 2011). Athanasiou et al. (2010) have reported that the response of dynamic environment is distinct than that of acclimation, and the dynamic environment is crucial for improving plants. The fitness of plants, grown under dynamic environment, has made them highly flexible, adaptive, and resistive to environmental stimuli (e.g., light, temperature, and humidity), and, thus, the controlled experiments might lack important components responsible for their robust and efficient photosynthesis (Cruz et al. 2016). Advantage in the fitness of *A. thaliana* under dynamic environmental conditions were reported by Kulheim et al. (2002); they found that mutants deficient in qE-type or ΔpH-dependent non-photochemical quenching of the excited state of Chl *a* had lower seed production under natural environment, but not when grown under controlled and static light conditions in growth chambers. Kulheim and coworkers suggested that since under natural conditions, plants are challenged with dynamic environments, many of their mechanisms may be up-regulated giving different results than under static conditions (see e.g., Kulheim and Jansson 2005; Rascher and Nedbal 2006; Murchie and Niyogi 2011). Further, Cruz et al. (2016) have used artificial phenotyping platform in which the dynamics of light intensity and light fluxes can be adjusted very close to that of the natural light. David Kramer and his associates have demonstrated the use of their platform and have investigated emergent phenotypes of several ChlF parameters including NPQ, Φ_{PSII} in a series of wild type and mutant lines. With growing attention in *plant phenomics*, Poorter et al. (2016) have discussed several experimental alternatives and options for facilitating stepwise translation of lab based results to field situations. We note that the adaptive significance of dynamic light, temperature and humidity with respect to important abiotic stresses, e.g., drought, low temperature and high salinity has thus far not been studied systematically. We assume that the lack of growth chambers simulating natural conditions may have been the main reason for the lack of such studies. However, the availability of light emitting diodes (LEDs) as light sources for plant cultivation makes it possible not only to control the light spectrum but also to obtain sinusoidal light waves in a growth chamber comparable to that in the sunlight. Further, with growing technical advancement, temperature, humidity and wind, can in principle, be also programmed to create artificial but reproducible environment close to what has been found in *nature*.

In order to further illustrate the importance of dynamic environment and its impact on plants, we show (in Fig. 5) diurnal variations in ChlF transients of *A. thaliana* accession *Tenela* (Te, origin Finland), grown in constant light (~100 µmol photons m^{-2} s^{-1}) but at different temperatures set to simulate a slow warming over the day, and progressive cooling during the night. While the large difference between curves recorded at 8 and 10 h reflects light adaptation, the difference between curves recorded at 10

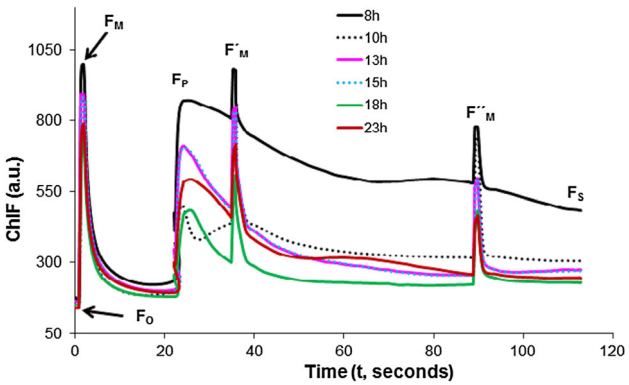

Fig. 5 Chlorophyll *a* fluorescence (ChlF) transients of *Arabidopsis thaliana* accessions "*tenela*" (Te) at different times (8, 10, 13, 15, 18 and 23 h) of the day. ChlF transients are average for whole plant rosettes measured by handy version of a fluorescence imaging system (Photon Systems Instruments). The temperature was 5 °C at 8 h (pre-sunrise); at 10 h, fluorescence transient was measured following an hour of ~100 µmol photons m^{-2} s^{-1}. Then, the temperature was slowly increased up to 18 °C throughout the day; finally, during the night, the chamber was slowly cooled (2 °C h^{-1}) to 5 °C. The day in the growth chamber started at 9 a.m. with the photoperiod, 12 h day and 12 h night. Each measurement of ChlF transients in the daytime was done with 5 min dark adaptation. The experimental protocol was modified from Mishra et al. (2014). F_O = minimum fluorescence measured right after dark-adaptation, F_M = maximum fluorescence measured by using saturating light (~1800 µmol photons m^{-2} s^{-1}), F_p = fluorescence peak (actinic light, ~50 µmol photons m^{-2} s^{-1}), F'_M and F''_M = fluorescence measured under saturating light at different time of actinic light, and F_S = steady state fluorescence

and 13 h, we suggest, results from temperature differences. These data reveal that the fluctuation of the environment has a significant impact on plant cold acclimation, and we propose that circadian rhythm of ChlF transients and associated ChlF parameters may indeed be an important non-invasive biophysical parameter, valuable for tracing these effects. For an early observation on circadian rhythm in ChlF of *Gonyaulax polyedra,* see Sweeney et al. (1979) and Govindjee et al. (1979).

Factors contributing to increase in plant stress (drought/cold) tolerance capacity are important

Among many biotic and abiotic stresses, responsible for losses in the yield, drought and cold are highly important; they also play an important role in determining geographical distribution of plant species (Hoffmann 2005). Different species develop different avoidance and tolerance strategies to survive unfavorable situations. In nature, gradual exposure of certain stresses enhances the tolerance levels of plant species to those stresses. For example, cold acclimation (CA, enhanced tolerance of plants to freezing stress by prior exposure to non-freezing temperatures) or drought acclimation (DA, enhanced levels of tolerance of plants to sever drought stress by prior exposure to mild/moderate levels of soil moisture deficit) provide to plants better survival strategy in tolerating harsh winters (Hannah et al. 2006; Mishra et al. 2011, 2014) or harsh drought conditions (Banik et al. 2016) as compared to that of their non-acclimated (NAC) counterparts. The acclimated plants achieve a new homeostatic state that is more compatible in efficiently maintaining their cellular integrity and photosynthetic activity under unfavorable conditions, and also in developing a capacity to better restore their proper cell function after the stress is removed (Moellering et al. 2010; Degenkolbe et al. 2012). In general, during harsh weather, e.g., sudden drop of temperature or in severe drought, membrane bound organelles (chloroplasts) or endoplasmic reticulum may become disorganized, proteins may undergo loss of activity or be denatured (Moellering et al. 2010; Skirycz et al. 2011; Perlikowski et al. 2016; Wan and Jiang 2016); further, in many cases, excess amount of reactive oxygen species (ROS) is produced leading to oxidative damage that may further cause senescence or even death to cells (Chen et al. 2015). Light is an essential component for full cold acclimation in *A. thaliana* (Catalá et al. 2011); systematic research efforts are needed to fully understand the physiological impact of the many factors contributing to achieve acclimation to drought or other stress factors in the dynamic environment on plants (Caldana et al. 2011; Juszczak et al. 2016).

Concluding remarks

We speculate that efficient exploitation of phenotying tools for the understanding of the function and physiology of both the root and the shoot systems, incorporating genetic factors into new varieties that can resist specific or multiple stresses and give high yield, targeted use of fertilizers and agrochemicals, and incorporation of new policies may lead us to *ever-green revolution or second green revolution*. This is expected to happen since the productivity may increase several fold even under low input, of e.g., water, and fertilizers (Lynch and Walsh 1998; Zhu et al. 2011; Ray et al. 2013; Pingali 2012; Kesavan and Malarvannan 2014). Undoubtedly, full characterization of plant phenomes, across all levels of organization, development and interactions with environment, is beyond the current technology. However, it is highly important that extensive research be done in this area with the ultimate goal of speeding -up agriculture productivity. We recommend that research community consider including the following in their work: (a) Identification of the molecular basis of highly important abiotic (drought/cold) avoidance or tolerance; we must ask how the process of acclimation is regulated and how it enhances the resistance capacity of plants; (b) Investigation of intermittent drought adaptation-induced modification in stress resistance capacity, and of the underlying photosynthetic mechanisms behind it all. Further, investigations on how plants' photosynthetic machinery and associated molecular, biochemical metabolism compete, when they are challenged with unprecedented changes in dynamic environmental stimuli (temperature rise and/or drop), are crucial for our understanding to achieve the goals discussed in this review.

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