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Plant Phenotyping Trends, Platforms and Initiatives

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The area of plant phenotyping is continuously progressing, with invasive, low throughput phenotyping methods being replaced by high-throughput, non-destructive methods. Rapid developments in non-destructive inexpensive sensors and imaging techniques over the last decade have revolutionized crop phenomics. Current implementations of non-destructive high-throughput phenotyping platforms include the use of sophisticated technologies such as: i) infrared thermography and imagery to scan temperature profiles/transpiration; ii) fluorescent microscopy/spectroscopy to assess photosynthetic rates; iii) 3D reconstruction to assess plant growth rate and structure; iv) light detection and ranging (LIDAR) to measure growth rates; v) magnetic resonance imaging and positron emission tomography to measure growth patterns, root/leaf physiology, water relations, and/or assimilate translocation properties; vi) canopy spectral reflectance for monitoring dynamic complex traits; vii) nuclear magnetic resonance for monitoring the structure of tissues, mapping water movements, and monitoring sucrose allocation; and viii) digital RGB imaging for recording data on various attributes of roots, shoots, leaves, seeds, and grains. The use of non-invasive sensors potentially enables the phenotyping of thousands of plants in one day, similar to the impact of high-throughput DNA sequencing technology in the field of plant genomics.

However, it also involves the generation of an unprecedented amount of complex data. Moreover, processing of images is not a trivial task and represents a bottleneck to using these tools to achieve high-throughput screening. A similar situation applies to the use of airborne sensors, where atmospheric and environmental conditions need to be measured and considered as cofactors in order to extract meaningful data. A variety of high-throughput phenotyping platforms exist and are currently used to phenotype different traits in almost all crop species. Some examples of these software /platforms include the Microsoft Excel-based macro, a tool called "LEAF-E" developed for analysing leaf growth parameters, "Zeppelin NT aircraft" as an experimental sensor platform used for remote aerial phenotyping, "Phenovator" and "GROWSCREEN FLUORO" for phenotyping large samples for photosynthesis and growth, and "TRiP (Tracking Rhythms in Plants)" for determining circadian period. Unmanned Aerial Vehicles have also been used successfully with various sensors to measure temperature and spectral vegetation indices associated to structural and physiological traits. Likewise, image-based phenotyping methods have been developed for measuring plant traits. For instance, imaging platforms have been used to record chlorophyll-a fluorescence, as indicators of cold tolerance and spikelet anthesis. Similarly, the "Phenoplant" platform successfully detected biotic and abiotic stresses using chlorophyll fluorescence. "PHENOPSIS" has been used to dissect plant responses to soil water deficit. The "Hyperspectral Absorption-Reflectance- Transmittance Imaging (HyperART)" system is used for noninvasive quantification of leaf traits (like disease severity).

In recent years, various state-of-the-art phenomics centers have integrated sensor platforms with precisely controlled conditions for cost-effective, high-throughput and deep plant phenotyping. Examples of such centers include the Plant Accelerator, High Resolution Plant Phenomics Centre, the Jülich Plant Phenotyping Centre, Leibniz Institute of Plant Genetics, Crop Plant Research in Germany, and the National Plant Phenomics Centre. In addition, information about plant phenotyping is being disseminated by the International Plant Phenotyping Network, which represents the world's major plant phenotyping centres. These centers utilize platforms designed primarily for phenotyping under controlled conditions, but efforts are being made to use develop relevant technologies for use under field conditions at both experimental and industrial scales. Various private companies offer custom, large-scale, high-throughput phenotyping platforms for both field and controlled environments.

Table. 2: Details of use of selected phenomics platforms for trait phenotyping in plants.

S. No	Platform /Technique/Software	Traits recorded /Approach used	Crop
Growth traits, phonological traits and physiological traits			
1	Light Curtain arrays (LCs)	Rapid determination of plant height and leaf area	Maize, barley, rapeseed and tomato
2	Microsoft Excel-based macro, a tool called "LEAF-E"	Analysing leaf growth parameters	Maize, Miscanthus spp. and *Brachypodium distachyon*
3	Zeppelin NT aircraft as an experimental sensor platform	Remote, aerial phenotyping with a mobile multi-sensor approach	Maize
4	Phenovator	For photosynthesis and growth	Arabidopsis
5	GROWSCREEN FLUORO	Growth and photosynthesis (PSII) efficiency	Arabidopsis
6	Digital still color camera under natural light	Chlorophyll content and Leaf nitrogen concentration	Rice
7	The image-based method	Flowering (spikelet anthesis)	Rice
8	TRiP (Tracking Rhythms in Plants)	Circadian period	Arabidopsis
Biotic and abiotic stresses (drought, heat, cold tolerance, salinity, nutrient-starving, UV light; low N-stress).			
10	Software for automatic RGB image analysis	Cold-tolerance (shoot biomass and photosystem II efficiency)	Pea
11	Imaging based methods	Chlorophyll-a fluorescence (ChlF)	Arabidopsis
12	Phenoplant	Chlorophyll fluorescence parameters impacted on leaves by a stress	Arabidopsis
13	Dual-mode microwave resonator	Water content of leaves and the ionic conductivity of the leaf	Potato, maize, canola and wheat
14	PHENOPSIS	Soil water deficit	Arabidopsis
15	Unmanned aerial platforms (UAP)	Low-nitrogen (low-N) stress tolerance	Maize
16	Automated video tracking platform	Resistance to aphids and other piercing-sucking insects	Arabidopsis and lettuce
17	Hyperspectral imaging (HSI)	Changes on the leaf and cellular level in plants during resistance reactions	Barley
18	Hyperspectral absorption-reflectance-transmittance imaging (HyperART)	Leaf traits (like disease severity)	Maize, barley, rapeseed, tomato
19	PHENODYN	Drought scenarios	Maize and rice
20	Terrestrial 3D laser scanning (TLS)	Canopy height	Maize, soybean and wheat

Phenotyping Platforms in Trait Phenotyping: Some Examples

The variety of high-throughput phenomics methods/platforms have been used for phenotyping of a variety of plant traits. For example using different phenotyping platforms, data has been recorded in high-throughput and automated manner for plant height, leaf growth parameters including leaf area, area of canopies, photosynthesis, photosynthesis efficiency, chlorophyll content, leaf nitrogen content and canopy height in different plant species including wheat, maize, barley, rice, pea, Arabidopsis, potato, canola, and soybean among others. Similarly, phonological traits like flowering (spikelet anthesis) in rice that is considered one of the most important but difficult to capture phenotypic characteristics and circadian period could be also estimated using highthroughput phenotyping platforms.

High-throughput methods have been also used to study plant responses to various types of abiotic stresses (drought, heat, cold tolerance, salinity, Nitrogen limitation, and UV light). Drought tolerance is considered one of the most important complex quantitative traits and many phenomics approaches have been used to understand the nature of drought tolerance. The approaches range from osmotic balance in hydroponics, to conveyer systems in glass house, to rainout shelters in the field. These encompass surveying of root system architecture in response to physiological parameters like water status, spectral imaging of shoot tissues, to evaluate yield under stress conditions in the field. These methods are based on imaging, robotics and computers which allow for automatic measurement of phenotypic traits for thousands of plants in a day in non-destructive manner. For instance, terrestrial 3D laser scanning (TLS) approach has been used to track the increase in canopy height of both monocot and dicot crop species under field conditions. Using this method, canopy height have been measured and it is expected that this method can be applied to detect the effects of plant stresses like drought, limited nutrient availability or compacted soil.

For biotic stress like insect pests, phenomics platform based on automated video tracking has been developed such as quantifying aphid feeding behaviour on leaf discs to assess the level of plant resistance. Such automated video tracking platforms can be used to screen large plant populations for resistance to aphids and other piercing-sucking insects in plants, as has been already tested on Arabidopsis and lettuce. Similar to abiotic stress phenomics, the area of disease phenomics is of immense interest for crop genetics and improvement. Large scale disease phenomics is expected to help in the accuracy and precision of detection and characterization of resistance reactions of crop plants against pathogens. Phenotyping for disease resistance in plants by visual assessment and rating is often time consuming and expensive. Therefore high-throughput platforms /methods developed like hyperspectral imaging (HSI) are considered promising non-invasive sensor techniques in order to accelerate and to automate classical phenotyping methods. This approach is important for evaluation of host-pathogen interactions over time and to discriminate among genotypes differing in susceptibility to disease. In another study, transient-induced gene silencing (TIGS)-based phenomics platform has been developed and used for testing of ~1,500 genes. This analysis identified 70 candidate genes showing significantly increase in resistance or susceptibility to fungal disease. Additional phenomics platforms are being developed for quantification of hyphal growth rates, higher level of automation, and expanding the host and pathogen range.

Conclusion

In summary, the phenomics platforms, methods, and tailored software have been used to record data in highthroughput fashion for a variety of traits in almost all important crops and the trait evaluation has also led to the genetic dissection leading to discovery of genes/QTLs for several traits including root system architecture traits, seed shape, osmotic tolerance, and biomass traits in crops like rice, wheat, barley and mustard. These successful examples suggests that phenomics holds great promise in uncovering most of the useful gene/QTLs governing complex quantitative traits in plants.

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