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PHENOMICS FOR BIOTIC STRESS RESISTANCE IN CROP PLANTS: A REVIEW

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ABSTRACT

Phenomics for biotic stress resistance is defined as the study of host plant resistance through automated trait analysis to generate phenotypic data. One of the greatest ways to control diseases that are both economically feasible and relevant over wide areas is to use resistant cultivars. To quantify phenotypic attributes using various sensors mounted on a platform, photos of an experimentally constructed field or a single plant must be taken. Additional image and data analysis is carried out using various software programmes. Plant resistance and pathogenicity or pathogen aggressiveness in a variety of genotypes analyzed through phenomics can be linked genomically with the identification of genes or Quantitative Trait Loci associated with resistance. Many automated tools that can be used for large-scale phenotyping to evaluate disease in field conditions have been developed and tested. This will help to meet the increasing demand for research into the development of new cultivars, yield increases, and disease-resistant cultivars that will support agricultural production in the future and ensure food security.

Key words: Phenomics, biotic stress, host plant resistance, large-scale phenotyping,

Introduction

By 2050, there will likely be nine billion people on the planet, meaning that present food production will need to double to feed everyone. (Joshi *et al.*, 2016) The challenge provided by many biotic and abiotic pressures connected to global food production has been worse recently due to decreasing climatic conditions. (Pereira 2016). Despite unfavourable environmental circumstances and a finite amount of cultivable land, the world's effort to feed the expanding human population necessitates a steady growth in crop production. (Furbank and Tester 2011).

Plant diseases and other productivity-inhibiting variables need to be researched and managed more thoroughly to stop annual losses in a variety of crops around the world. The most sensible and cost-efficient

method of disease control is the cultivation and use of resistant cultivars, which can be applied across wide regions, are extremely effective against infections when properly managed, and do not endanger human health or the environment. Therefore, among the numerous control strategies, using resistant cultivars is always the first choice. In many circumstances, this is sufficient to manage the losses brought on by the primary diseases that impact a particular crop, and in other situations, it can help with additional supplementary phytosanitary measures. As a result, disease resistance is regarded as a "key agronomic trait" of novel cultivars, and any breeding effort should prioritize the development of these traits. Therefore, through the integration of genotyping and phenotyping, a thorough understanding of the intricate relationships between plants, pathogens, hosts, and humans is essential to the success of genetic breeding.

To keep up with the advancements in high throughput genotyping, a technology race has been taking place in recent years, leading to the development of large-scale techniques and tools for high-throughput phenotyping.

Phenotyping has been transformed by high-throughput phenotyping technology, which is expanding quickly. It uses automated sensing, data collection, and analysis, and depends on automated trait analysis to produce phenotypic data. Plant breeding efforts have accelerated with the use of high-throughput phenotyping, which allows for the screening of a large number of plants at different phenological stages. This means that early on, desired features can be quickly screened for, removing the need to wait for plant maturity in the field. It may be applied in controlled and natural environments both in the lab and the outdoors. Plant performance may be quickly assessed in the field, which promotes a thorough life cycle assessment using less harmful methods. Furthermore, in high-throughput facilities with controlled environments, data recording is enhanced and fewer replications are needed. This review focuses on large-scale phenotyping of plant disease resistance through phenomics, which combines breakthroughs in imaging techniques, automation through software, and plant science to characterize plant responses to numerous environmental conditions.

Components of Phenomics for Plant Resistance and Pathogen Virulence

The following are the fundamental components of phenotyping, which can be implemented to any type of crop, disease, resistance type, and either an outdoor or indoor environment (Fig. 1).

1. Choice of imaging technique and sensor type
2. Choice of platform
3. Experimental design used
4. Choice of image processing software
5. Data management and choice of storage and transmission software

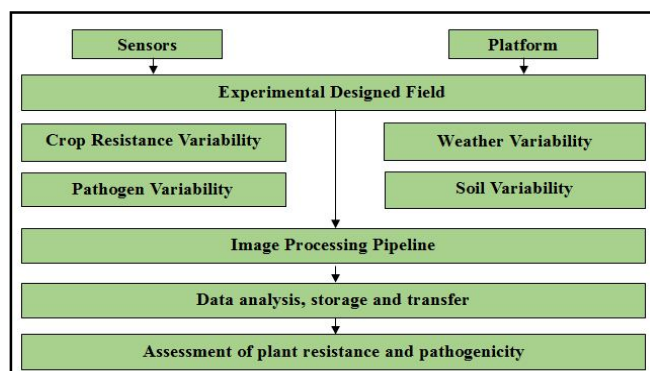


Fig. 1: Components of Phenomics.

Imaging Techniques

Electromagnetic waves are regarded as detection information carriers. Plants in good health interact differently with electromagnetic radiation than do plants in diseased conditions. Each constituent of plant tissues and cells has absorbance, reflectance, and transmittance characteristics that are unique to particular wavelengths (Li *et al.*, 2014). Usually, imaging techniques for phenomics include RGB imaging, fluorescence imaging, thermal imaging, spectroscopic imaging, and techniques involving 3D imaging (MRI, PET, CT, LIDAR, and stereo vision) (Table 1).

Visible Light Imaging

Visible images are derived from digital images and are meant to resemble human vision, which is sensitive to visible light wavelengths (400–750 nm). The responses that can be estimated through visual imaging includes, such as shoot biomass (Golzarian *et al.*, 2011), yield traits (Duan *et al.*, 2011), panicle traits (Ikeda *et al.*, 2010), imbibition and germination rates (Dias *et al.*, 2011), leaf morphology (Hoyos-Villegas *et al.*, 2014), seedling vigour (Walter *et al.*, 2012), coleoptile length and biomass at anthesis (Richards *et al.*, 2010), seed morphology (Chern *et al.*, 2007), and root architecture (Iyer-Pascuzzi *et al.*, 2010).

Fluorescence Imaging

Fluorescence imaging is the main method used for disease detection in leaves. The first metabolic processes impacted by disease infection are those that occur during the transition from photosynthetic to respiration and the derivation of nutrients from flow. Fluorescence imaging is the primary method used to monitor this process. Light that is released at certain shorter wavelengths when radiation is absorbed is known as fluorescence. Fluorescence imaging is frequently employed in a controlled environment because modulated fluorescence demands a significant amount of power for quick illumination. To track the impacts of plant diseases and identify early stress responses to abiotic and biotic variables before a drop in growth can be observed, fluorescence imaging can quantify photosynthesis (Li *et al.*, 2014). Swarbrick *et al.*, (2006) utilized chlorophyll fluorescence to study the resistance response of barley leaves infected with *Blumeria graminis*. Throughout a vulnerable encounter, photosynthesis decreased gradually on the entire leaf. By employing chlorophyll fluorescence imaging to screen sugar beet lines with varying susceptibilities to *Cercospora beticola* infection, Chaerle *et al.*, (2007) demonstrated that variations in fluorescence intensity were quantified between susceptible and

Table 1: Types of images, and examples of their application used in several studies for quantifying the severity of diseases.

Image	Patho systems
RGB	▪ Barley-<i>Erysiphe graminis</i> (Newton 1989) ▪ Maize-<i>Fusarium</i> spp. (Todd and Kommendahl 1994) ▪ Potato- (Niemira <i>et al.</i>, 1999) ▪ Maize-maize streak virus (Martin <i>et al.</i>, 1999) ▪ Oats-<i>Puccinia coronata</i> (Díaz- Lago <i>et al.</i>, 2003). ▪ Vine-<i>Plasmopara viticola</i> (Boso <i>et al.</i>, 2004) ▪ Rye and triticale-<i>Magnaporthe oryzae</i> (Maciel <i>et al.</i>, 2013) ▪ Sugarcane-<i>Cercospora longipes</i> (Patil and Bodhe 2011) ▪ Cotton-Bacterial angular (Sugiura <i>et al.</i>, 2016) ▪ Cotton-Ascochyta blight (Bock <i>et al.</i>, 2010 and Wijekoon <i>et al.</i>, 2008)
Hyperspectral	▪ Apple tree-<i>Venturia inaequalis</i> (Delalieux <i>et al.</i>, 2007) ▪ Sweet potato-(<i>Cercospora</i>, <i>Erysiphe</i>, <i>Uromyces</i>) (Mahlein <i>et al.</i>, 2012) ▪ African oil palm-<i>Ganoderma boniense</i> (Lelong <i>et al.</i>, 2010) ▪ Wheat-<i>Puccinia striiformis</i> f. sp. <i>tritici</i> (Arora <i>et al.</i>, 2013)
Thermal infrared	▪ Pumpkin-<i>Pseudoperonospora cubensis</i> (Oerke <i>et al.</i>, 2006) ▪ Vine-<i>Plasmopara viticola</i> (Stoll <i>et al.</i>, 2008) ▪ Apple tree-<i>Venturia inaequalis</i> (Oerke <i>et al.</i>, 2011) ▪ Olive tree-<i>Verticillium</i> (Calderon <i>et al.</i>, 2013)
Chlorophyll fluorescence	▪ Sweet potato-<i>Cercospora beticola</i> (Chaerle <i>et al.</i>, 2007) ▪ Millet-<i>Puccinia substriata</i> (Costa <i>et al.</i>, 2009) ▪ Bean plant-<i>Xanthomonas fuscans</i> pv. <i>fuscans</i> (Rousseau <i>et al.</i>, 2013) ▪ Wheat-<i>Fusarium</i> spp. (Bauriegel and Herppich 2014)
Visible near-infrared	▪ Sugarcane- Orange rust (Apan <i>et al.</i>, 2004) ▪ Wheat- Head blight (Bauriegel and Herppich 2014)
Near-infrared	▪ Barley- Powdery mildew (Kuska <i>et al.</i>, 2015)
Short wave infrared	▪ Maize- <i>Phaeosphaeria</i> leaf spot (Adam <i>et al.</i>, 2017) ▪ Wheat- Powdery mildew (Delalieux <i>et al.</i>, 2005) ▪ Wheat- Leaf rust (Wahabzada <i>et al.</i>, 2015)

resistant plants. Burling *et al.*, (2010) used fluorescence imaging to investigate variations in the degree of wheat cultivar resistance to *Puccinia triticina* and showed that the quantum yield of non-regulated energy dissipation in PSII can be used to distinguish between them. A more noticeable variation in parameter values.

Thermal Imaging

Reduced rates of photosynthesis and transpiration are common responses to biotic stressors. Therefore, using thermal imaging to remotely sense leaf temperature can be a dependable method of identifying changes in a plant's physiological state. It makes it possible to see infrared radiation, which shows the temperature distribution of an object. The most widely utilized wavelengths for thermal imaging are 3-5 μm and 7-14 μm , while the sensitive spectral range of thermal cameras is 3–14 μm (Li *et al.*, 2014).

Imaging Spectroscopy

The interaction of solar radiation produced with plants is used in plant imaging spectroscopy. A significant amount

of incoming energy is reflected by leaves in the near-infrared (NIR; 700-1200 nm) due to scattering inside the leaf mesophyll. The absorption bands in the infrared region are utilized to characterize different water indices and multispectral and hyperspectral measurements are frequently employed to estimate the canopy water content as an indicator of water condition (Li *et al.*, 2014). As demonstrated by the findings of an investigation by Sabatier *et al.*, (2013) used near infrared reflectance spectroscopy as a high-throughput screening tool for pest and disease resistance in a sugarcane breeding programme, the hyperspectral measurement makes it a promising method for determining the severity of damage caused by insects. Using GreenSeeker, Arora *et al.*, (2014) assessed vegetation indices for the precision phenotyping of quantitative stripe rust reactions in 120 genotypes of Indian wheat. The results show that temporal ground-based NDVI is the most useful method for examining the quantitative rust reaction, with a significant regression coefficient ($r^2 = 0.63$) between the NDVI data and the area under the disease progress curve.

3D Imaging for Plants

Currently, light detection and ranging (LIDAR) sensors, also known as laser scanners, and stereo vision are the most often utilized image sensor technologies for 3D plant mapping.

High-resolution topographic maps and extremely precise estimations of the height, cover, and canopy structures of plants can be produced using LIDAR technologies. Moreover, laser scanning has potential in plant pathology and allows the assessment of photosynthetic efficiency when paired with fluorescence (Li *et al.*, 2014). Stereo vision was used by Mizuno *et al.* (2007) for wilt detection and Takizawa *et al.*, (2005) to construct 3D models of plants.

Tomographic Imaging by MRI, PET, or CT

MRI is the acronym for “Nuclear Magnetic Resonance Imaging,” a technique that utilizes nuclear magnetic resonance to create images and identify signals coming from ^1H , ^{13}C , ^{14}N , and ^{15}N .

A nuclear imaging method called PET (positron emission tomography) creates a three-dimensional image or picture of a working process. It finds pairs of gamma rays that are released by a radionuclide that releases positrons. Labeled chemicals like ^{11}C (Jahnke *et al.*, 2009), ^{13}N (Kiyomiya *et al.*, 2001), or ^{52}Fe (Tsukamoto *et al.*, 2009) can be imaged noninvasively to determine their distribution.

A technique called X-ray computed tomography creates tomographic images of particular sections of the scanned item using computer-processed X-rays. It may create a 3D image of an object’s interior from a large number of 2D radiographic images recorded around a single axis of rotation. (Li *et al.*, 2014). For high throughput plant phenotyping, however, the tomographic imaging systems still have poor throughput, and additional improvements in picture segmentation and reconstruction are needed.

Platforms

A phenotyping platform is characterized as one that can photograph a minimum of hundreds of plants every day (Fahlgren *et al.*, 2015) based on the installation of several desired sensors that can screen both individual plants and entire fields. Due to patent protection, the underlying hardware and software of the large phenotyping platforms, which are primarily produced by commercial companies cannot be altered to accommodate particular research requirements. (Czedik-Eysenberg *et al.*, 2018). As a result, a variety of self-developed and customized commercial phenotyping platforms are being

introduced. Phenotyping platforms can be classified under three scenarios, (1) aerial based (2) ground-based proximity based (3) greenhouse based (Li *et al.*, 2021). Fig. 2 shows the different types of platforms that can be used under these conditions.

Experimental Design

There are no particular references for the most common experimental designs utilized in large-scale phenotyping. Numerous studies have emphasized the need to design trials to maximize the accuracy of the phenotypic data (Araus and Cairns 2014; Fiorani and Schurr 2013; Cobb *et al.*, 2013; Poorter *et al.*, 2012). According to field research, certain designs like the augmented block design (ABD), incomplete block design (IBD), and randomized complete block design (RCBD) as well as other adjustments could improve phenotyping effectiveness.

In the early stages of a breeding programme, when there is still a significant amount of material to be analyzed and primarily, when there is minimal propagation material, ABD is most frequently utilized. On the other hand, RCBD is more frequently employed in the later phases of breeding programmes when more trustworthy results regarding the examined treatments are needed, in addition to having enough propagation material to do multiple replicates. An incomplete Block Design is utilized when the block size in the greenhouse or field is smaller than the total number of plants or treatments (Paternelli and Resende, 2015).

Imaging Processing Pipeline

Imaging analysis can be done using various open-source software or otherwise. The imaging processing pipeline is software-specific but fundamentally includes

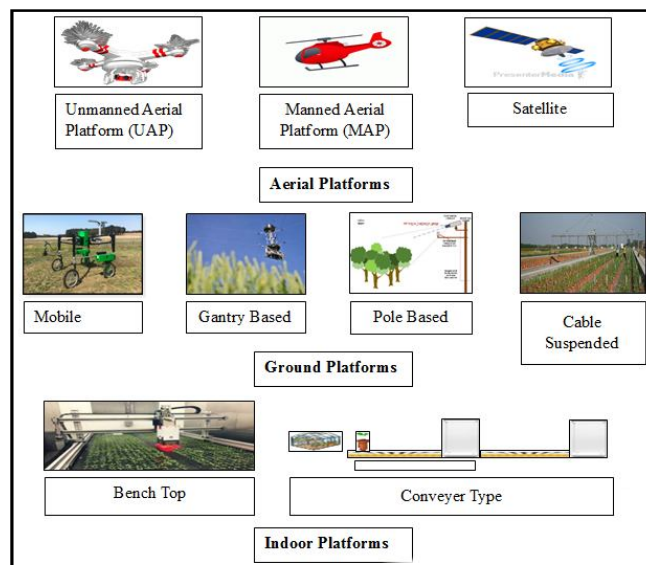


Fig. 2: Types of platforms used in phenomics.

the following operations. The process of processing images begins with image retrieval, which includes importing the photos from the database into the programme used for image analysis and aligning them using reference points created by image stacking. All of the photos of a single object taken at various times are made into an image stack. The second phase is called image preparation, and it involves applying filters to reduce noise or sharpen images. The image is separated into objects of interest and objects that should be excluded from the study during the image segmentation process. Binary images are segmented images where all pixels set to 0 are scored as 0, and all pixels that relate to the object of interest are rated as 1. Noise reduction, or morphological operations, is the process of repairing the binary pictures' flaws. The subsequent stage, known as picture composition, involves assembling the separate parts of an object to form the whole. Quantification of an object's area, height, width, and other dimensions is a necessary step in the image description process. The process of colour classification entails removing the subject, in this case, the plant, from the original RGB picture. As a result, using the colour information from the original image, the plant portion may be separated into various sections, which can then be quantified to quantify various morphological characteristics (Singh and Singh 2015). Image processing software used in several studies for quantifying the severity of diseases, ASSESS, Image Pro Software, JL Genias, Sigma Scan, Sigma Scan Pro software, Skye-Probetech, Soft Imaging Systems GmbH, Microsoft C compiler, Image09, Visual C++, QUANT, ER Mapper, EASI/PACE, ENVI, ERDAS Imagine, GRASS GIS, IDRISI, PG Steamer, TNT Mips, Image Intelligence TM Suite, Remote View (Bock *et al.*, 2010).

Data Management and Storage

The development of phenotyping methods in the last few years has produced datasets with data from various sensors at various scales and organizational levels. These datasets can be mined or pooled in meta-analyses to produce new insights if they are made available to the scientific community. However, getting them accessible is a big problem for the plant phenomics community.

Dealing with the massive volume of data produced by phenotyping devices and facilities is the first issue. Big data presents a challenge because of its complexity, which can be summed up as follows:

- (i) The Volume given the exponential increase in data obtained through phenotyping techniques;
- (ii) The Variety of data due to the multiplicity of data sources;

- (iii) The Velocity, given the need to give scientists quick and effective tools for visualizing and analyzing large amounts of experimental data;
- (iv) The Value, given that phenomics experiments are costly and nearly impossible to repeat precisely; and
- (v) The Veracity, which is tied to the requirement to track data such as the steps, Calibration of sensors, parameter settings, and methods that have been employed to produce a particular result (Roitsch *et al.*, 2019). Wilkinson *et al.* (2016) introduced the FAIR principle to organize and integrate a large amount of data, allowing data to be found and reused across various persons or groups. FAIR stands for identifiable, reusable, findable, and available.

The second issue is the requirement of facilitating database interoperability among diverse scientific communities. Steps in this direction, allowing data interoperability while maintaining security and privacy, include the farmer-focused Open Ag Data Alliance and the public-private alliance IAA (International Agro-informatics Alliance) at the University of Minnesota (Gustafson *et al.*, 2017).

In the big data-driven agricultural world, one of the main worries is the quality of the data. According to Shakoor *et al.*, (2019), there is general agreement that “garbage in” of primary data quality leads to “garbage out” of final data quality since low-quality positive images utilized in machine learning models could produce inaccurate predictions. For future phenomics research

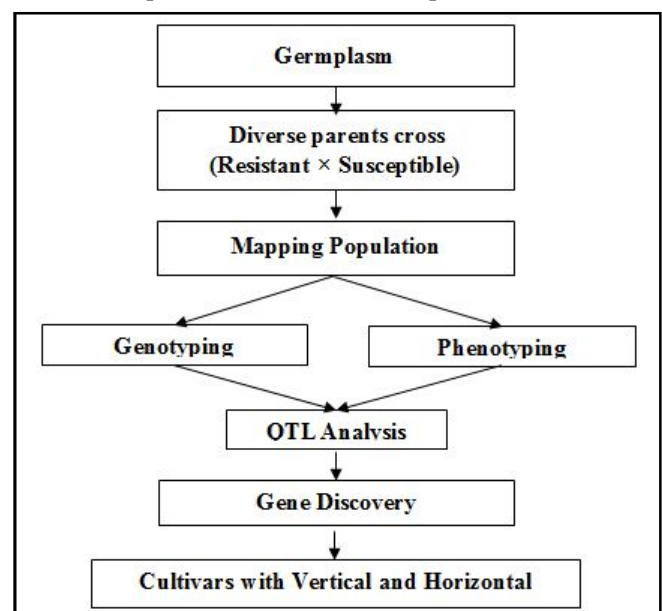


Fig. 3: Flowchart of the activities of a breeding program for phenomics for producing resistant cultivars

projects, quality protocols need to be established and standardized. Quality assurance and checks also become more crucial for sensor accuracy and consistency.

Identification of genes associated with resistance using plant phenomics

The creation of a germplasm bank with a fairly wide genetic basis that contains sources of susceptibility and resistance to the primary diseases impacting the crop in question is the first step in the identification of genes associated with resistance. Crosses between parents who are susceptible to and resistant to one or more diseases give rise to mapping populations like F₂, RIL, and NIL. Genomics may make it simple to determine these populations' genotypes. Different phenotypical features are quantified and analyzed individually in the phenomics process. The identification of the relationship between plant genes (genotype) and resistance (phenotype) using QTL analysis can therefore be applied to molecular marker-assisted genotype screening and selection of genotypes exhibiting vertical or horizontal resistance (Fig. 3).

Conclusion and Future Thrust

Phenomics studies of plant diseases have a bright future ahead of them. These studies seek to better understand the intricate interactions between humans, environment, and pathogens, as well as to identify genes and QTL associated with pathogenicity and resistance. With this knowledge, genetic breeding programmes aimed at producing new cultivars resistant to a variety of crop diseases can be accelerated. Despite the challenges involved, phenotyping is crucial since methods, platforms, sensors, tools, software, and devices are already available for the large-scale identification and quantification of diseases in the lab, greenhouse, and field. But significantly more widespread adoption, popularisation, and distribution of these instruments are needed. To fully benefit from using the range of phenomics technology presently available, plant breeders with holistic perspectives and experience, particularly in phytopathology, epidemiology, genomics, and breeding need to be further involved. This will help enhance food sustainability.

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