



Quantitative and qualitative phenotyping of disease resistance of crops by hyperspectral sensors: seamless interlocking of phytopathology, sensors, and machine learning is needed!

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Determination and characterization of resistance reactions of crops against fungal pathogens are essential to select resistant genotypes. In plant breeding, phenotyping of genotypes is realized by time consuming and expensive visual plant ratings. During resistance reactions and during pathogenesis plants initiate different structural and biochemical defence mechanisms, which partly affect the optical properties of plant organs. Recently, intensive research has been conducted to develop innovative optical methods for an assessment of compatible and incompatible plant pathogen interaction. These approaches, combining classical phytopathology or microbiology with technology driven methods — such as sensors, robotics, machine learning, and artificial intelligence — are summarized by the term digital phenotyping. In contrast to common visual rating, detection and assessment methods, optical sensors in combination with advanced data analysis methods are able to retrieve pathogen induced changes in the physiology of susceptible or resistant plants non-invasively and objectively. Phenotyping disease resistance aims different tasks. In an early breeding step, a qualitative assessment and characterization of specific resistance action is aimed to link it, for example, to a genetic marker. Later, during greenhouse and field screening, the assessment of the level of susceptibility of different genotypes is relevant. Within this review, recent advances of digital phenotyping technologies for the detection of subtle resistance reactions and resistance breeding are highlighted and methodological requirements are critically discussed.

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Current Opinion in Plant Biology 2019, **50**:156–162

This review comes from a themed issue on **Biotic interactions**

Edited by **Rebecca S Bart** and **Ken Shirasu**

<https://doi.org/10.1016/j.pbi.2019.06.007>

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Resistance of crop plants to fungal pathogens

The most sustainable tool in crop protection is breeding of resistant plant varieties. In general, disease resistance is characterized as an observed phenotype in a specific environment on which a pathogen (fungi, bacteria, or viruses) is not able to cause a disease. The cultivation of resistant crops can reduce the applications of plant protection products and therefore reduce negative impacts of agriculture on the environment.

In crop plants a multifaceted defence system is involved during pathogen attack [1,2]. Several functional processes can underlie disease resistance and different phases in the infection or developing stage of a pathogen can be affected, respectively. Among these, pathogen resistance can be related to mechanical barriers (e.g. the wax layer, cuticle, or cell wall) hindering the penetration or proliferation of a fungal pathogen [3]. In specific interaction types, a hypersensitive response (HR) — described as the rapid development of cell death at and around infection sites occur [2]. Both inhibit growth of the pathogen into the plant tissue, and thus reducing the production of infectious structures, such as spores or conidia. Genetically, incompatibility or resistance is often driven by a specific interaction between the pathogen's avirulence (avr) gene loci and alleles of the corresponding plant disease resistance (R) locus [4].

Plant breeding is a dynamic process, considering the performance of varieties in differing environments, in which the integration of technological progress is an important aim. Continuous research is necessary to increase the genetic resistance pool of crop plants without compromising yield or quality parameters [5]. Occasionally plant pathogens overcome effective plant resistances, due to recombination or spontaneous mutations — facilitated by a high and fast reproduction rate of especially fungal pathogens. Resistance is never permanent and boom and bust cycles have been observed in the past especially for pathogens like rust or powdery mildews in cereals, exhibiting on gene per gene interaction to their host plant [6]. Furthermore, globalization, human activities and climate change promote favorable conditions for emerging pathogens [7,8]. In such a dynamic environment, a couple of steps

make the breeding process time and labor intensive and therefore it is not possible to react ad hoc to pathogen developments [9,10]. Most prominently, there is a bottleneck since the capacity and the throughput during the traditional phenotyping process by human experts is still comparatively low [11,12^{*}]. Detection, characterization and integration of host–pathogen interaction during the breeding process are still time and labor intensive and there is an increasing demand to accelerate these steps significantly.

Within this context, recent innovations and developments for plant breeding are most welcome. Besides new breeding technologies such as CRISPR-Cas [13,14], digital phenotyping [15,16^{*}] will significantly form and influence plant resistance breeding.

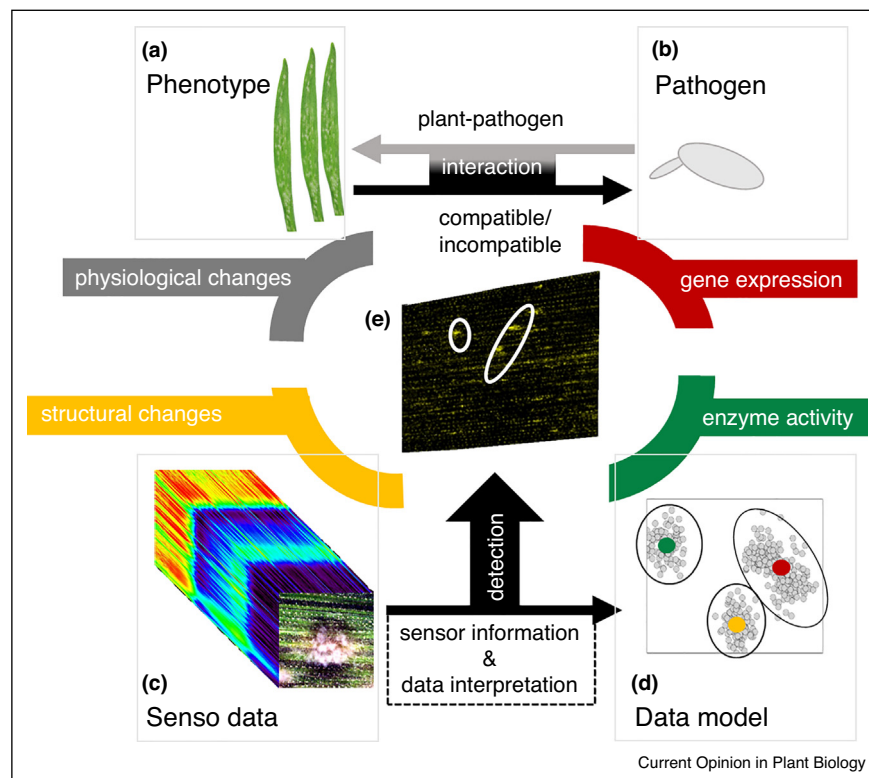
Digital phenotyping to uncover incompatible host–pathogen interaction

The assessment of incompatible host–pathogen interactions or resistance reaction is compared to the assessment of plant diseases significantly more complex and challenging (Figure 1). Resistance reaction — such as hypersensitive reactions or lignification — take place on the

cellular level and are highly subtle. Multiple changes occur at differing time points and cause changes in the physiology and biochemistry of a plant. The complexity increases even more, since interaction among a plant's genotype and the pathogen are highly individual as well. Therefore, these mechanistic processes are difficult to phenotype. Recently, different studies provided evidence, that these subtle processes of host–pathogen interaction in turn influence the optical properties of plants (reviewed in Mahlein *et al.* [17^{**}]). In general, changes in reflectance and optical properties of plants resulting from plant pathogens can be explained by impairments and changes in the leaf structure and highly specific chemical compositions of the tissue during pathogenesis. During the last decade, several non-invasive sensors were established to enable a deeper view into the interaction types [18,19^{**}]. Besides the non-invasive manner of these technologies, it is a further advantage that image assessment and analysis can be performed in a fraction of time compared to common methods and in high throughput [20,21].

Especially hyperspectral imaging and non-imaging sensors are valuable tools to be implemented in breeding

Figure 1



Complexity of functional and mechanistic processes during compatible and incompatible host (a)–pathogen (b) interaction, causing changes in optical properties of plants. Detection and characterization of resistance reactions on the leaf or cellular level is realized by hyperspectral imaging technologies (c) combined with artificial intelligence and machine learning algorithms mining in a data model (d) at massive scale can be realized. Visualized in this example is a false color image of a barley leaf section with small scale HRs (e).

routines on different scales: from the tissue to the canopy level. For plant breeding, two differing aspects are of interest — a quantitative approach and a qualitative approach. Quantitative assessment, for example, the disease severity of a leaf pathogen is relevant in breeding trials for rating the behavior of genotypes, lines or varieties under disease pressure. Imaging technologies provide the potential to detect plant diseases and enable a quantification in percentage of the diseased leaf area [22]. A recent study of Thomas *et al.* [23] correlated disease severity, assessed by hyperspectral imaging in an automated Mini-Plot screening greenhouse to disease rating by human experts and scores from official variety trials with high accuracy. It was possible to quantify powdery mildew symptoms automatically through a combination of Simplex Volume Maximization and Support Vector Machines in a data-driven analysis routine. The qualitative assessment or characterization of a specific resistance reaction is more complex. Until now only few researchers integrated sensor technologies for an assessment (i.e. disease scoring) and characterization (i.e. understanding the underlying mechanisms of plant pathogen interaction) of resistance reactions of plants to pathogens. There are advantages and disadvantages on the assessment scale which have to be considered critically. Future research also has to consider improved sensor platforms and vehicles for field and

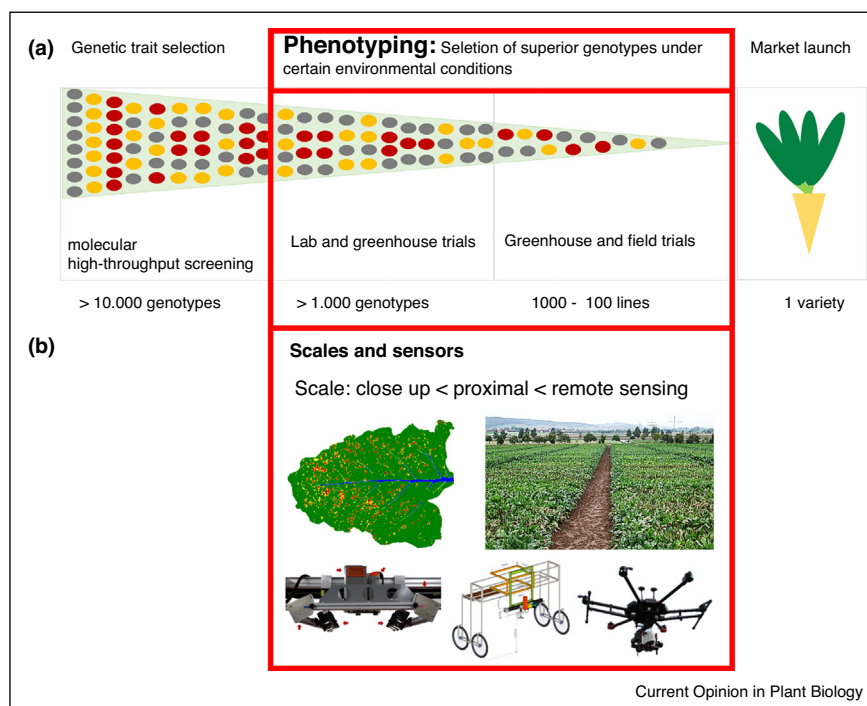
high-throughput applications. It is expected, that new developments in the field of robotics will speed up the integration into agricultural practice.

Equipment and measurement setup: current potential and limitation of technology

From the technical perspective, four different kinds of hyperspectral sensor technologies are available: push broom scanner, whisk broom scanner, filter-based sensor and non-imaging sensor [20,24]. These sensors provide different pro and cons: push broom and whisk broom scanners have a comparatively high spatial and spectral resolution, but the measuring process is time-consuming and the subsequent data processing is more complex since objects are scanned line by line. Filter-based system measure an object waveband by waveband. The measuring process is comparatively time consuming and vignetting effects cause often partially noisy data within an image. Snap-shot system are desirably, but so far existing systems have either a non-acceptable low spectral and/or spatial resolution. The required calibration routines do not exist and therefore the spectral signal is not plausible.

The choice of the sensor depends on the application and sensor platform and often requires a well thought balance between spatial resolution, information content of the measurement, the time available and finally the costs

Figure 2



Integration of digital phenotyping technologies into the breeding process **(a)**. Optical sensors can operate on different platforms from close up over proximal to remote sensing **(b)**. Evaluation of crop plants is performed in laboratory, greenhouse or field trials **(a/b)**. During these processes, a qualitative assessment of resistance reactions or a quantitative evaluation of disease severity can be realized to evaluate resistance of crop plants to pathogens.

(Figure 2) [25,26]. In breeding gardens, for example a larger area coverage is often required, which may be balanced by using fast and cheap sensors. Here UAVs, tractor-based or stationary platforms are often used [27,28,29*,30,31]. For understanding functional relationships in host–pathogen interactions, specialized high resolution sensors are often more appropriate [32,33].

For the detection and assessment of plant resistance reactions on a small-scale hyperspectral microscopes enable the analysis and assessment of individual host–pathogen interactions in detail [17**]. In the study of Kuska *et al.* [33], the hyperspectral reflectance of powdery mildew, diseased barley leaves and resistant leaves were recorded in time series measurements. The results revealed a characteristic development of the spectral pattern for the different host–pathogen interactions. By applying a machine learning approach, the authors established the identification of powdery mildew diseased leaves one to two days before symptoms became visible. In addition, race-specific resistance of barley against powdery mildew was analyzed and the spectral reflectance characteristics were linked to hypersensitive reactions [34]. A similar system was used for an improved phenotyping approach of *Cercospora beticola* spots on sugar beet [35]. In accordance with the hyperspectral reflectance pattern, the authors showed that the *Cercospora* leaf spot symptoms have different functional regions, which depend on the genotype of the sugar beet and linked to susceptibility, resistance or tolerance. Going deeper into the details of the genetic background of investigated genotypes, Leucker *et al.* [36] showed that HSI can be used to analyse the effect of QTLs and their resistance effect against *C. beticola*. Until now the hyperspectral microscope systems are limited for lab applications, because of the low throughput and highly reduced field of view of below 1 cm. However, for specific applications a trade-off among spatial details and assessment throughput is acceptable. Several studies successfully demonstrated the use of digital phenotyping on crop or canopy scale. Pretorius *et al.* [37] for instance consistently detected QTL regions to stripe rust in wheat populations from spectral data, indicating that remote sensing technologies can be applied in genetic mapping of stripe rust resistance on the field scale. Further studies integrated hyperspectral imaging successfully on the leaf scale for proximal sensing and phenotyping, for example, Septoria leaf blotch (STB) [38]. Yu *et al.* [39*] investigated the same pathosystem on the canopy scale. Both studies developed quantitative metrics for infection levels of STB and were able to link wheat genotypes to differing susceptibility. As reflected by Rebetzke *et al.* [40**] following this approach a greater diversity in larger population sizes can be captured combining an improved repeatability and robustness in resistance screening.

Furthermore, many systems cannot utilize the ‘whole’ electromagnetic spectrum (ultraviolet, UV; visible, VIS;

near infrared, NIR; shortwave infrared, SWIR). The SWIR spectral range can provide additional insights into host–pathogen interactions and may be included in the measurement portfolio [41]. A hyperspectral imaging system that uses two push broom cameras (from 400 to 2500 nm) enabled the assessment of the resistance status and resistance mechanism of different grapevine cultivars to *Plasmopara viticola* infestation on the leaf scale [42]. The HyperArt is also a platform that can measure the wavelengths from 400 to 2500 nm by using two hyperspectral cameras [43]. The system provides a simultaneous assessment of spectral reflectance and transmittance, which can be used to calculate the light absorption [44].

The benefit of the above-mentioned approaches is manifold, but what are the critical aspects, and what is demanded to provide these technologies for breeding practice? So far, a significant number of researches were performed for phenotyping disease resistance. All of these works focussed on a specific host–pathogen system; opportunities to transfer the results and insights to other systems are limited. Measuring setups are prototypes, tailored to the specific task. Here especially the scale of assessment (cellular, leaf, single plant, canopy) influences the implemented technologies. The complexity of the sensor setup is high and experts can only perform running the platform. These experts are trained scientists with differing background and so far: learning by doing still characterizes the situation.

The measuring environment and scale significantly determine the opportunities and limitations of hyperspectral imaging for plant resistance phenotyping. Today, still the throughput and precision correlate negatively. A high precision investigation of lignification as a resistance response is possible sample by sample using a complex hyperspectral imaging microscope. Here the measuring conditions are stable and the reproducibility under artificial illumination is high. For field phenotyping, environmental conditions are not stable and sun light intensity, wind, rain and abiotic stress to plants influences the data quality [reviewed in Ref. 24]. Therefore, several platforms use a curtain and artificial illumination to exclude the influence of natural light. A huge potential lies in new and innovative platforms from robotics [17**] and UAV technologies. But here often the weight of the sensor which can be carried and the focal plane limits the use of specific sensors [29*].

For a high throughput, online routines are desirable; that is, the data assessment and data analysis are performed immediately after each other on the platform computer. This is so far limited computing capabilities of on-board computers and the complexity of machine learning algorithms, therefore often offline approaches, with data assessment and decision or management action are separated are still the standard.

A positive aspect which has been respected during the last decade is the establishment of several high-quality research centers for phenotyping technologies. Among them (to name just some) are the Australian Plant Phenomics Facility, Adelaide Australia, the Donald Danford Center in St. Louis, Missouri USA, the Excellence Initiative PhenoRob at the University of Bonn and Research Center in Jülich, Germany and the e-Agri Sensors Center at the University of Manchester UK. In addition, traditional breeding centers such as the CIMMYT, Mexico or the IPK Gatersleben, Germany invest in infrastructure and establish research groups within the topic. This research and investment are crucial for bridging the step into breeding routine.

From complex data to meaningful interpretation: linking spectral signatures to individual interactions

From a technical, biological and functional perspective, optical sensors and hyperspectral imaging are future oriented technologies for resistance breeding. However, there are several critical aspects and challenges: Data are often collected at massive scale — up to several TeraBytes, if plants are monitored over time. Manual labelling is often prevented as subtle changes are hard to locate or their rare occurrence is covered by the natural variability. Sophisticated algorithms and powerful hardware systems have to be tailored to a specific application, a transfer among crop species and pathosystems is not always possible. Furthermore, experts with complementary skills and scientific backgrounds such as phytopathology, plant breeding, informatics or electrical engineering have to integrate their knowledge to yield ‘easy to interpret’ insights from data representations with a biological meaning. The available toolbox contains many new algorithms and data analysis routines [45,46^{••}]. Methods from the field of artificial intelligence (e.g. data mining, machine learning, and pattern recognition) and computational sustainability already contribute to provide solutions for plant phenotyping, even in high-throughput setups [46^{••},47,48]. Recently, fast data-driven approaches for mining sensor data in resistance screening are a significant step forward to meet the demands of interpretability and scalability [49,50[•]]. Archetypal signatures, characteristic for interaction types can be assessed while avoiding the risk of losing information and without time-intensive data labelling [51]. The presented methods have one limiting factor in common. First off all, parameters cannot be generalized and have to be individually adapted to data characteristics and features of interest. Secondly, the ratio of training sample size and required model complexity is decisive for the optimization process. These training data are needed either for training the model and/or afterwards for model evaluation. The annotation process has to be done by human experts and is time consuming. Therefore, many prediction models in resistance phenotyping by hyperspectral imaging face the problem of limited training data. To compensate they may rely

on available models pre-trained on millions of images, which in plant science literally do not exist. Here we need more open source data and collaboration among researchers. Huge data machines such as Google or Facebook easily can produce billions of training data for a specific class, but a database of spectral resistance patterns would help to overcome this limitation.

A future task is to integrate genotype–phenotype data for a holistic view on disease resistant genotypes based on digital phenotyping, genome sequencing data, to extensive transcriptomic, methylomic and metabolomic data [52,53]. Results and approaches are of utmost interest. However, so far only few pilot studies attempted to include phenotype to genotype data to explore host–pathogen systems. Kuska *et al.* [54] linked enzyme activity profiles to multispectral data of barley–powdery mildew interaction in high-throughput for the identification of biomarkers. Similarly, Arens *et al.* [41] correlated metabolic markers and hyperspectral data for a screening of resistant sugar beet to *Cercospora* leaf spot. In the context of data integration, we expect a significant step forward within the next five years.

Conclusion

Digital phenotyping technologies as an integrative approach of plant pathology, sensors and artificial intelligence support the investigation of crop resistance against plant pathogens. So far, there is not one standard measuring setup or data analysis approach, which can be generalized for the differing tasks in plant resistance phenotyping. An integration of genotype–phenotype data for a holistic view on disease resistance will provide new insights into host–pathogen interaction. Future challenges include the development of scale and environmentally independent features. Hyperspectral, scale independent fingerprints or spectral libraries of specific resistance mechanisms support the idea of an accelerated digital phenotyping in high throughput.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work could be carried out due to the financial support of the German Federal Ministry of Education and Research (BMBF) within the scope of the competitive grants program ‘Networks of excellence in agricultural and nutrition research – CROP.SENSE.net’ (Funding code: 0315529), junior research group ‘Hyperspectral phenotyping of resistance reactions of barley’ and due to financial support of the Daimler and Benz foundation. This manuscript is dedicated to Professor Heinz-Wilhelm Dehne, an important mentor who passed away to early on the 23th of May 2019.

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