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BluVision Macro - a software for automated powdery mildew and rust disease quantification on detached leaves.

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Software

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Summary

Powdery mildews and rusts are in the Top 10 of the major fungal pathogens in plant pathology (Dean et al., 2012) (Huerta-Espino et al., 2011) (Zeder, 2011). The effect of powdery mildews on crop yields can amount to 40% of harvested grain (Jarvis et al., 2001). Besides being an agriculturally important pathogen, the powdery mildews of wheat and barley are important models for studying the plant-pathogen interactions (Douchkov et al., 2014). Wheat leaf rust and stripe rust are two other fungal pathogens, frequently causing epidemics with up to 70% yield losses (Chen, 2005) (Huerta-Espino et al., 2011) in combination with a decreased grain quality (Duveiller, Singh, Mezzalama, Singh, & Dababat, 2012).

Crop protection against pathogens is mostly provided by the application of chemical agents (Popp, Pető, & Nagy, 2013), however, many of them have detrimental effects on non-target species (Stanley & Preetha, 2016). Therefore, the trend is towards reducing the pesticide application and development of alternative and integrated protection methods (Mzoughi, 2011). The most sustainable method for crop protection appears to be the use of natural genetic resources and breeding for disease resistance (Gómez, Rodríguez-Hernández, Moury, & Aranda, 2009). Plant breeding is as old as the domestication of the first agricultural plants (>10000 years) (Zeder, 2011). The "Green revolution" (Hazell, 2009) of the 1950-1960 and the more recent "omics" revolution (genomics, proteomics or metabolomics, etc.) (Yadav, 2007) introduced many new approaches and technologies but the observation methods of the breeders remained mostly unchanged. Recently, new high-throughput observation methods for Phenomics (Bilder et al., 2009) were introduced, thus providing the fundament for another breeding revolution. However, the powdery mildews and rusts are, as the majority of the plant pathogens, microscopic organisms in the initial and most critical stages of the infection process. Phenotyping of these early stages was significantly held back by the lack of technology for high-throughput phenotyping on a macroscopic and microscopic scale.

To solve this problem, we have developed the BluVision Macro framework aimed to allow strictly quantitative assessment of disease and host responses on a macroscopic level. The system consists of a hardware part – the Macrobot (Lueck et al., 2020) - a multimodal imaging station and robotized sample magazine/loader, and the BluVision Macro software, described in this article. The system is designed to work with samples placed in microtiter plates (MTP), which are well-established standard in biology and medicine. The loading of the MTPs to the imaging station and the image acquisition is fully automated. The system uses a 14-bit monochrome camera (Thorlabs 8050M-GE-TE) at a resolution of 3296×2472 px. The illumination is based on narrow bandwidth isotropic LED light sources (Metaphase Exolight-ISO-14-XXX-U) with 365nm (UV), 470nm (blue), 530nm (green) and 625nm (red) peak wavelength. For each plate monochrome images in all illumination wavelengths are acquired

separately and stored in 16-bit TIFF image files. Additionally, a background illumination image is taken and used for the separation of the foreground and background.

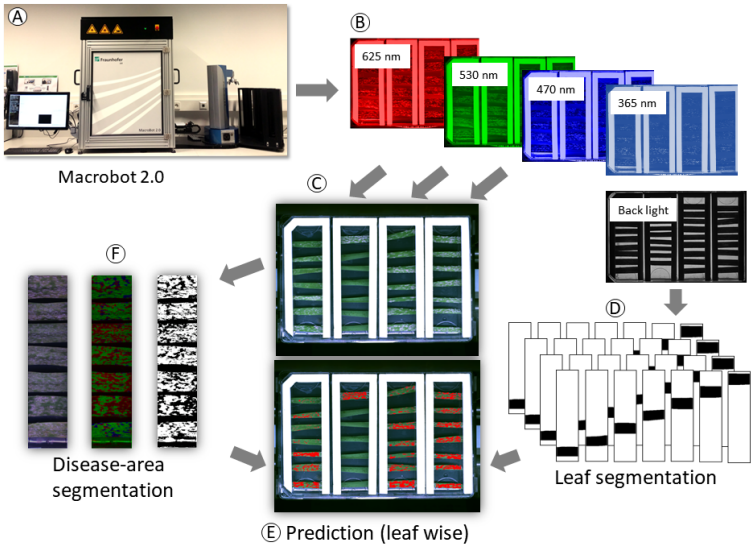

Figure 1: Macrobot image acquisition and analysis workflow.The 14-bit monochrome camera of the Macrobot (A), acquires images in 5 different illumination scenarios (channels) for each plate (B). The red (625 nm), green (530 nm), and blue (470 nm) channels are combined into an RGB composite image (C), which is further segmented for detection of the infected area of the leaves (F). The UV (365 nm) and the backlight channel, are used for the leaf segmentation (D). Combined prediction for infected area and leaves serves to calculate the infected area as a percent of the leaf surface (E).

The image analysis pipeline currently contains software modules for powdery mildew of barley and wheat (*Blumeria graminis* f. sp. *hordei* resp. *tritici*), wheat stripe rust (*Puccinia striiformis* f.sp. *tritici*), wheat leaf rust (*P. triticina*), and will work without major modification for barley leaf, stripe rusts and probably other leaf diseases with a similar appearance. Phenotyping of other disease and non-disease related phenotypes will require development of dedicated modules. The system is running in production mode and generates phenotyping data for powdery mildew and rust disease resistance screens at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, and the Julius Kühn-Institute (JKI) in Quedlinburg, Germany.

Installation

The macrobot software can be installed with *Ananconda* and *pip* and was built and tested in the Microsoft Windows environment. It requires Python 3.8 or higher, numpy (Walt, Colbert, & Varoquaux, 2011), scikit-image (Walt et al., 2014), opencv-python (Bradski, 2000), pytest and jinja2.

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Additional information

This paper is dedicated in memory of Patrick Schweizer.

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