High throughput quantitative phenotyping of plant resistance using chlorophyll fluorescence image analysis

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Résumé en anglais	Background In order to select for quantitative plant resistance to pathogens, high throughput approaches that can precisely quantify disease severity are needed. Automation and use of calibrated image analysis should provide more accurate, objective and faster analyses than visual assessments. In contrast to conventional visible imaging, chlorophyll fluorescence imaging is not sensitive to environmental light variations and provides single-channel images prone to a segmentation analysis by simple thresholding approaches. Among the various parameters used in chlorophyll fluorescence imaging, the maximum quantum yield of photosystem II photochemistry (Fv/Fm) is well adapted to phenotyping disease severity. Fv/Fm is an indicator of plant stress that displays a robust contrast between infected and healthy tissues. In the present paper, we aimed at the segmentation of Fv/Fm images to quantify disease severity. Results Based on the Fv/Fm values of each pixel of the image, a thresholding approach was developed to delimit diseased areas. A first step consisted in setting up thresholds to reproduce visual observations by trained raters of symptoms caused by Xanthomonas fuscans subsp. fuscans (Xff) CFBP4834-R on Phaseolus vulgaris cv. Flavert. In order to develop a thresholding approach valuable on any cultivars or species, a second step was based on modeling pixel-wise Fv/Fm-distributions as mixtures of Gaussian distributions. Such a modeling may discriminate various stages of the symptom development but over-weights artifacts that can occur on mock-inoculated samples. Therefore, we developed a thresholding approach based on the probability of misclassification of a healthy pixel. Then, a clustering step is performed on the diseased areas to discriminate between various stages of alteration of plant tissues. Notably, the use of chlorophyll fluorescence imaging could detect pre-symptomatic area. The interest of this image analysis procedure for assessing the levels of quantitative resistance is illustrated with the quantitation of disease severity on five commercial varieties of bean inoculated with Xff CFBP4834-R. Conclusions In this paper, we describe an image analysis procedure for quantifying the leaf area impacted by the pathogen. In a perspective of high throughput phenotyping, the procedure was automated with the software R downloadable at http://www.r-project.org/ [11]. The R script is available at http://lisa.univ-angers.fr/PHENOTIC/telechargements.html [12].
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