Developing image analysis for phenotyping quinoa-downy mildew interaction

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Introduction

Quinoa (*Chenopodium quinoa*), is a crop with favourable nutritional properties and evaluated for cultivation in numerous countries. Fungal diseases like downy mildew limit grain yield and the development of resistant varieties is therefore a central goal of quinoa breeding.

Phenotyping responses to downy mildew (***Peronospora variabilis***) in quinoa has proven difficult for standard RGB imaging because of the interference caused by various shades of green and pink colors that the different quinoa genotypes express on their leaves Figures 1 and 2.

Objectives

- Develop an image analysis protocol able to discriminate diseased from healthy tissue on quinoa leaves infected with downy mildew
- Phenotype for severity and sporulation with a multispectral image analysis approach

1. Phenotypic response to downy mildew by visual scoring

Severity is the percentage of leaf area covered by a lesion on the adaxial side. This can be orange, yellow or pink depending on the genotype (Fig. 1)

Sporulation is the amount of sporulation present on top of the lesion(s) and measured in percentage. It is assessed on the abaxial side of the leaf (Fig. 2)

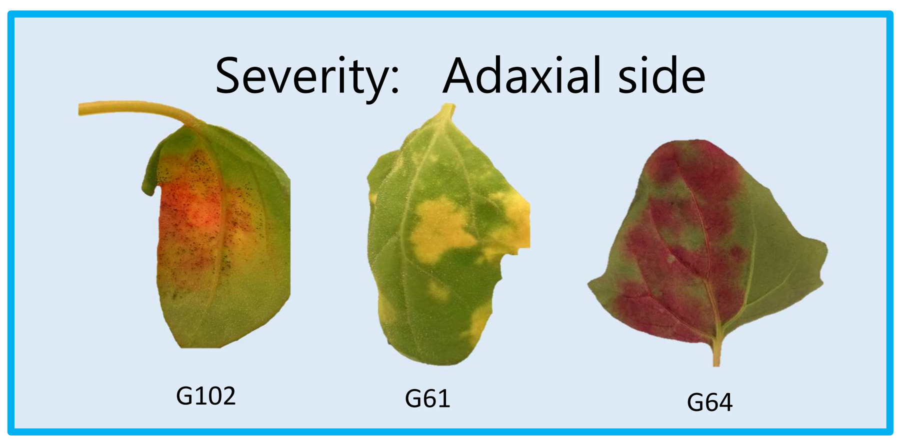

Figure 1. Severity symptoms on adaxial side of the leaves

Figure 2. Sporulation prescence on abaxial side of the leaves

2. Multispectral image analysis

We performed multispectral imaging with the VideometerLab 4 unit, where a light integrating sphere ensures homogenous illumination of the subject (Fig. 3). Every image stack acquired consists of 19 distinct image bands, each illuminated with its own color ranging from UVA at 365 nm to NIR (near infrared) 970 nm. Each pixel in the image is ~41 µm in both directions. Every layer in the stack is 2192 by 2192 pixels.

Severity model for image analysis

For the adaxial (top) side of the leaf from genotype G9 (Fig. 4) we see chlorosis is clearly present (A) in the sRGB (a "normal camera" image created from the humanly visibly bands in the image stack. (B) and (C) shows two individual bands from the multispectral stack, with blue at 490nm (B) and yellow at 570nm (C). After an initial annotation of what is considered healthy plant tissue and what is chlorotic, a model is build, by first transforming (D) the information from the 19 bands (the layers in the image stack) by nCDA (normalized Canonical Discriminant Analysis) into a value range representing the pixels through the stack. This is then used for a segmentation (E and F) that can be applied to all the images - all the cultivars and genotypes - to obtain a quantification of the percentage of the leaf that has only chlorotic tissue (E, in yellow) at 68.0% for this particular leaf, or including the spore-covered area (F) in red and covering 18,9%, the chlorotic (yellow) at 68%; combined spore and chlorotic area 75,8%.

Sporulation from image analysis

In the abaxial (the bottom) side of the leaf, the sporulation is clearly visible in the imaging (Fig. 5) for this genotype (G9) in the sRGB image (A and in B the lower left part enlarged). It is though, not so easy to detect in the individual bands in the visible spectrum, here exemplified with the blue band (490nm) (C). Moving into the NIR bands at 780nm (D and in E the lower left part enlarged) we see the spores clearly. Using this information (only the clearly dark grey spore clusters) enabled us to distinctly segment the spore pixels (F) and quantify the area, which for this leaf is 12.5% spores (shown in yellow), excluding chlorotic areas. Also, here the spore annotation was kept more conservative than for the adaxial image analysis. An annotation that instead covered also the not-so-dark-grey pixels (pixels are bigger than the individual spores) resulted in a spore estimate at ~23% (not shown here).

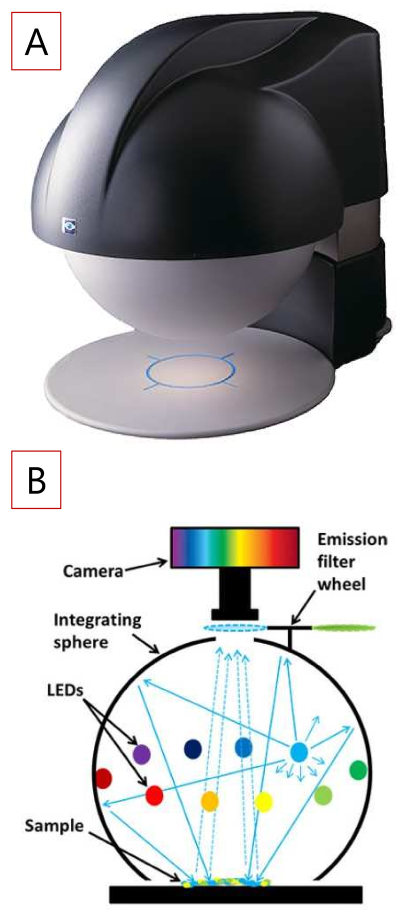

Figure 3. VideometerLab 4.
(A) The imaging unit.
(B) The illuminating diodes (here only one per color shown) are inside the sphere and illuminate the object one color after another.

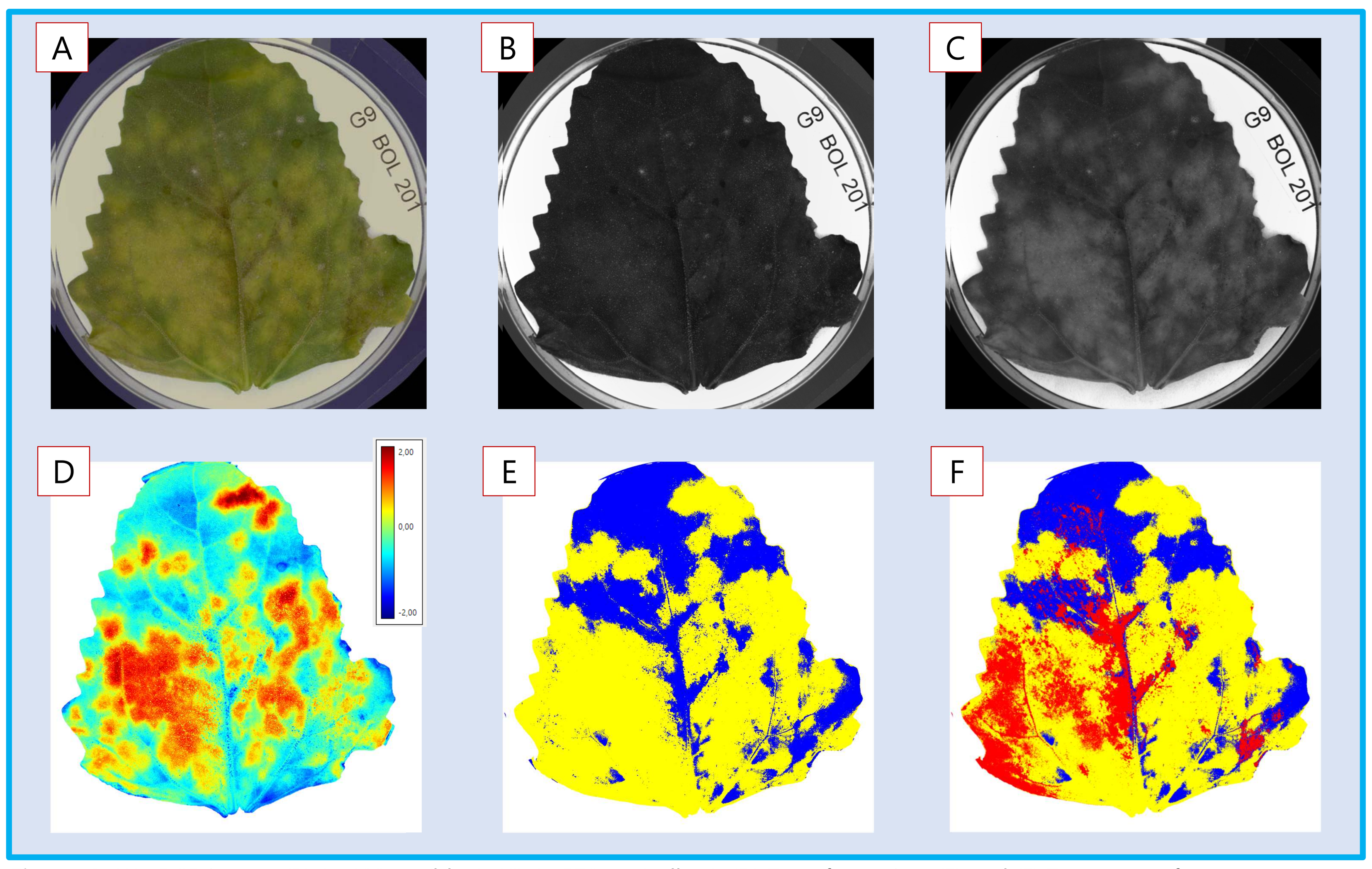

Figure 4. (A) sRGB image. (B) At 490nm (blue). (C) At 570nm (yellow). (D) Transformation. (E) and (F) Two types of segmentation with quantification.

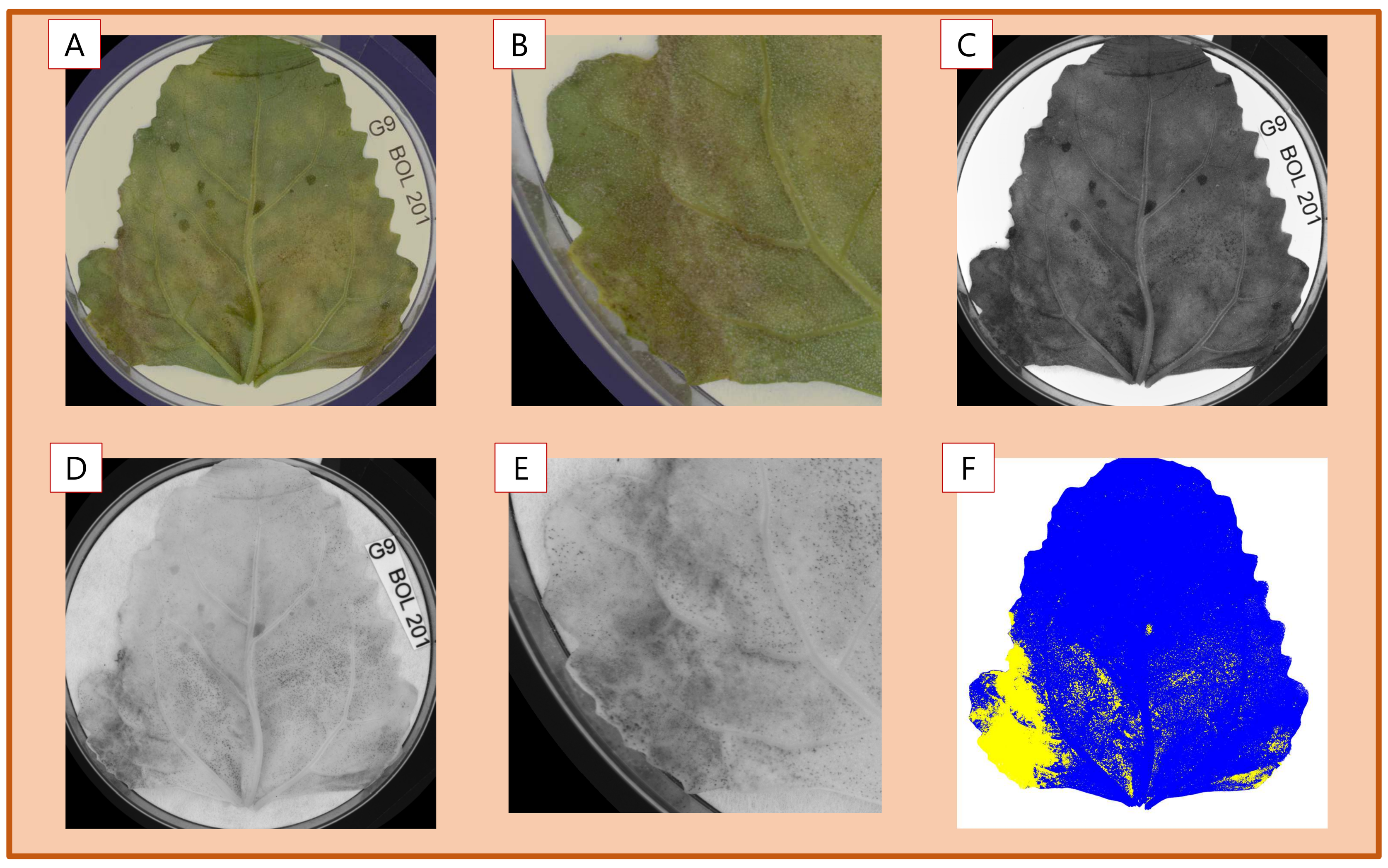

Figure 5. (A) sRGB image. (B) At 490nm (blue). (C) At 570nm (yellow). (D) Transformation. (E) Segmentation with quantification.

Results

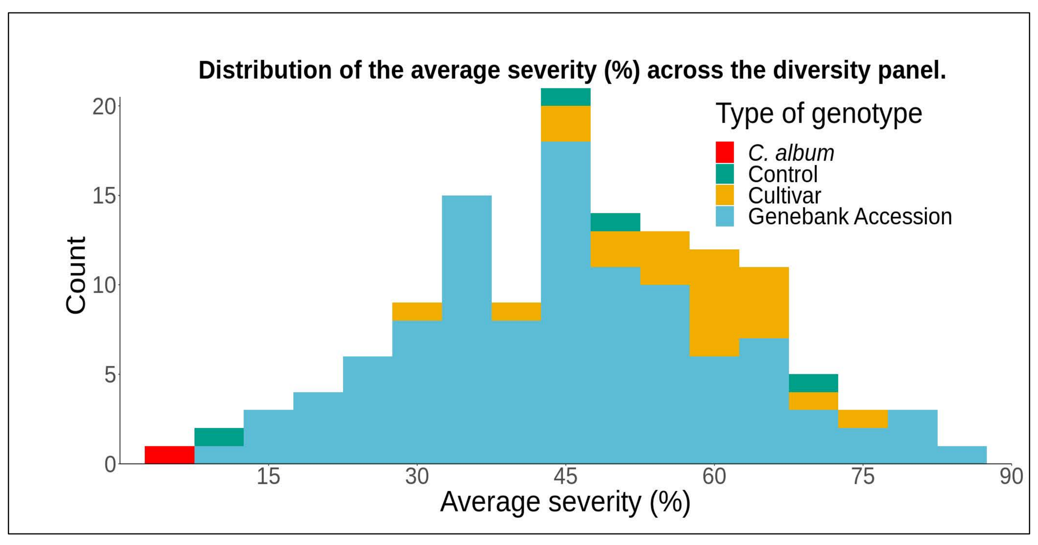

Fig 6: Distribution of the average severity (%) on leaves of 133 genotypes

Responses to downey mildew disease were phenotyped from an association panel of 132 quinoa genotypes. Three experiments, each with four blocks with replicated controls and unreplicated genotypes, were set up. Large variation in the response to ***P. variablilis*** plus a highly-significant effect of the genotype on the interaction was found (p-value = 1.18 x 10^{-18}) Fig. 6.

We compared results above with image-based phenotyping. We are developing our algorithms to cover the whole spectrum of responses. In this poster we have shown examples of imaging from one leaf corresponding to G9. Results summarised on table 1 come from the average of values obtained from 10 leaves belonging to separate plants coming form different blocks and experiments.

Table 1. Manual and image base phenotyping for quinoa-downy mildew interaction

Genebank Accession G9	% Average severity	% sporulation
Image base phenotyping	83	3,4 (conservative algorithm and chlorosis not included)
Visual Scoring	85 (Chlorosis 69%- Sporulation 19%)	77

Conclusions

- We have learned that multispectral imaging is a strong phenotyping tool when applied with a strict protocol and good documentation.
- Quantification of images are based on the application of algorithms which are fed by experience and knowledge of the disease responses. This accounts for a better approximation on pixel annotation for sporulation, hyphae germination, penetration and area covered by lesion as well as recognition of healthy tissue.
- Algorithms that can be applied to lesions produced by all quinoa genotypes-downy mildew interactions are being developed with encouraging results.

*Colque-Little C, Correa M, Andreasen C, Buchvaldt D, Schmid, KJ, Genetic contributions to tolerance for downy mildew pathogen *Peronospora variabilis* in a South American panel of quinoa. Poster presented at International Symposium of the Society for Plant Breeding; 2020 Feb 11-13; Tulln, Austria