Comparative Analysis of Two Reliable Systems for Assessing Gray Mold in Strawberry Fruits

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BACKGROUND

- Gray mold causes financial losses for strawberry growers, reducing fruit yield and quality.
- *Botrytis cinerea* is a broad host range necrotrophic fungus causing soft-rotting symptoms (Elad *et al.*, 2007).
- *Botrytis* infection can be difficult to manage due in part to quiescent infection.
- Early detection of infected strawberry fruits, before appearance of symptoms, allow accurate diagnosis (Abdel Wahab, 2015), especially before fruit export.
- Quantitative real time PCR (qPCR) application was developed in the second half of the 1990s and has offered the ability of simultaneous detection and quantification of DNA based on nucleic acid sequences and concentrations (Zeng *et al.*, 2006; Abdel Wahab and Younis, 2012).
- QPCR technology has many advantages: quantitative properties, high sensitivity and specificity, which make this technique suitable for routine usage and disease management decisions (Postollec *et al.*, 2011)
- Use of remote sensing for detection of crop diseases is based on interference with photosynthesis and physical structure of the plant, and the absorption of light energy, thus altering the reflectance characteristics of the plants.
- Reflectance spectra of vegetation, measured in the visible and infrared regions, contain information on plant pigment concentration, leaf cellular structure, leaf moisture content (Borengasser *et al.*, 2001), and plant anomalies (Wu *et al.*, 2008).
- Although *B. cinerea* disease was early detected used VIS-NIR reflectance spectroscopy at asymptomatic stages, hyperspectral technology with narrow spectral bands is crucial for providing additional information for the spectroscopic characteristics for any plant organ tissues.

OBJECTIVE

The purpose of this study is to use a dual reliable system for early detection of gray mold in strawberry fruits which may have quiescent infection. This would demonstrate the strengths and weaknesses of each tool: qPCR (molecular system) and spectroradiometer (spectral reflectance system) to discriminate between healthy and infected strawberry fruits under laboratory condition. Such dual system can be potentially used in parallel providing alternative synergic verification for early diagnostic.

MATERIALS & METHODES

★ Plant sample and DNA preparation

In order to early detect the existence of the fungal pathogen *B. cinerea* from strawberry fruits (Var. Festival & Sweet Charlie), ten fruits were taken from each plant variety and tested by qPCR and spectroradiometer. DNA extraction from strawberry fruits was performed using Plant Genomic DNA Miniprep Kit, according to the manufacturer manual.

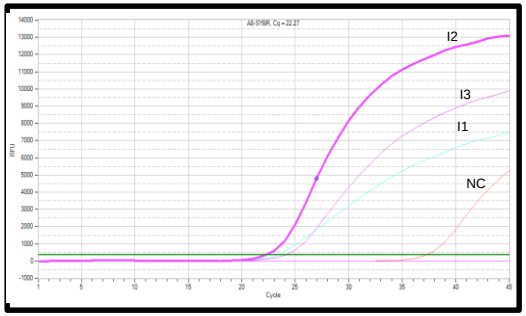
★ Quantitative Real-time PCR amplification (qPCR)

Specific *B. cinerea* primers targeting the ribosomal region between 28S and 18S genes (intergenic spacer, IGS) reported by Rigotti *et al.* (2002) were used: Bc424f: 5'-GCT TCC CCC GTA TCG AAG A-3'; Bc491r: 5'-CGA ACG GCC AGG TCA TCT-3'. Amplification program was done as the following: 10 min at 95°C, followed by 40 cycles of three-steps amplification run at 95°C for 30 s, 55°C for 45 s and 72°C for 45 s for amplifying IGS region, and 10 min at 95°C, followed by 40 cycles of three-step amplification run at 95°C for 30 s, 45°C for 45 s, and 72°C for 45 s for amplifying actin gene.

★ Spectroradiometer measurements

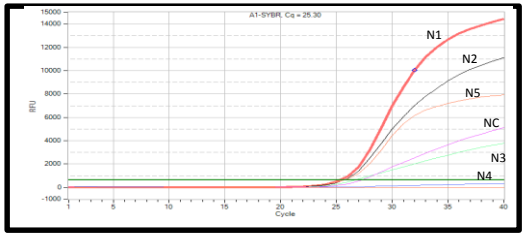
Spectroradiometer was used indoor to measure the reflection of twenty samples of strawberry fruits under investigation. Measurements were carried out in a full optical spectral range (Visible – Near Infrared – Short Wave Infrared) starting from 350 nm to 2500 nm. The protocol used for the collection of spectral data is based on measuring radiance from a Spectralon® panel. A designed probe was attached to the instrument's fiber-optic cable to be used to ensure standardized environmental conditions for reflectance measurement. Measurements were performed using the contact prop. Five spectra of each strawberry fruit were obtained. The mean of the five spectra was then determined to provide a single spectral value that represents spectral reflectance pattern or spectral signature for each sample. The results of the spectral reflectance measurements were then compared with that of qPCR test to evaluate the reliability of them for grey mold detection in strawberry fruits.

RESULTS

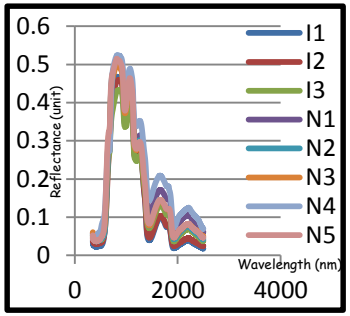


★Amplification of genomic DNA of symptomatic and symptomless *Botrytis* infected fruits with SYBR-Green shows a distant amplification start point between the tested fruits: I2, I3 and I1 and the negative control, NC, indicated that these tested fruits have *Botrytis* infection.

★Similar cycle threshold values were found between these infected fruits indicated a little variation in infection degree between them.



★Amplification of genomic DNA of healthy strawberry fruits with SYBR-Green shows similar cycle threshold values between the tested fruits: N1 - N2- N3 - N4 - N5 and that of the negative control, NC, indicated that these tested fruits have not any *Botrytis* infection.



★Analysis of the spectral reflectance of strawberry fruits indicated that VNIR and SWIR-2 were the best spectral zones to differentiate between healthy and infected strawberry fruits.

★Healthy fruits (N1, N2, N3, N4, N5) showed higher spectral reflectance than that of infected ones (I1, I2 and I3) throughout the whole spectral zone range.

SUMMARY

Gray Mold is a serious constraint in strawberry production worldwide. Early detection of infected strawberry fruits, before appearance of symptoms, allow export decision. Two useful alternative systems, molecular and remote sensing technologies have been used for monitoring large plant samples at a single time point. Similar measurement values were observed between some tested strawberry fruits and negative control using both qPCR and spectroradiometer tests confirmed that these tested fruits have not any *Botrytis* infection. Likewise, infected fruits showed similar values with that of the positive control using the previous two tools confirmed their infection with *B. cinerea*. The spectral reflectance of all tested strawberry fruits demonstrated that VNIR and SWIR-2 were the best spectral zones to differentiate between healthy and infected strawberry fruits. Moreover, healthy fruits showed higher spectral reflectance values than that of infected ones throughout the whole spectral zone range. Results of this study suggest the parallel potential usage of laboratory remote sensing and qPCR to monitor gray mold in symptomless infected fruits. Thus it will be useful for assessing the quiescent infection before export.

CONCLUSION

★ The long-term goal of this research is to develop a fast screening technique that can accurately detect and quantify *Botrytis* infections in symptomless strawberry fruits.

★ The two comparative detection systems: molecular and spectral techniques were evaluated to detect the *Botrytis*-infected samples from different infection levels of strawberry fruits.

★ The current study demonstrated that both techniques: qPCR and spectroradiometer are reliable assays for early diagnosis of gray mold in strawberry fruits under laboratory condition.

★ Both two systems have differentiated between the healthy and infected strawberry fruits and demonstrated similar measurement values when using test control.

★ Generally, the qPCR cycle threshold and the spectral reflectance values of healthy fruits were higher than those of infected ones along with the whole sample collection.

★ The results investigated that VNIR is the best spectral zone which could discriminate between healthy and infected fruits due to *Botrytis* infection effect on the mineral and fulvous composition in the fruits, while SWIR-2 is the best spectral zone to distinguish between fungal patterns within the infected fruits.

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