Monitoring of second overtone of water absorbance bands reveals hypersensitivity response from virus infected plants

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Introduction

Study of hypersensitivity reactions of plants after they have been invaded by harmful viruses is immensely important to ensure the sustainability in agriculture. Recently, near-infrared (NIR) spectroscopy has become popular in closely monitoring a variety of bio-systems by means of a new water and perturbation spectroscopy called "aquaphotomics".[1] However this area of science has not yet been widely applied in plant biology. Soybean mosaic virus disease (SMV) is an important disease that adversely affects both soybean yield and oil content. Multiplication of the virus inside a host plant from "healthy" up to "diseased" is a complex biological scenario, which could allow possibility for the electro-magnetic spectrum to detect corresponding water structural forms associated with the disease.[2] This study describes the ability of closely monitoring hypersensitivity reactions from SMV infected plants by means of the changes in absorbance of two SWNIR (short wavelength NIR) water bands.

Materials and methods

Twenty plants of Soybean *(Glycine max)* were grown in greenhouse conditions. Ten plants were inoculated with SMV strain B, eight days after germination. The ELISA reference test confirmed that only the inoculated plants tested positive for the disease. Ten leaf spectra per plant were acquired every two days for 24 days, until the disease symptoms became visually apparent on the leaves. A NIR spectrometer FQA-NIR Gun (Shizuoka Shibuya Seiki, Hamamatsu, Japan) was used to acquire leaf spectra from 730 nm to 1100 nm at 2 nm intervals in reflectance mode.

The data analysis was performed by Pirouette (3.11 Infometrix, Inc WA, USA). The wavelengths most sensitive to the SMV disease were illustrated by the standard deviation (*SD*) profiles calculated from the spectra from diseased and healthy plants at each wavelength. The SIMCA class distance between the two groups was obtained as a time series, following the progression of the disease. Estimates of free (S_{0es}) and bonded (S_{2es}) water were calculated, based on the leaf absorbance (Log $1/R$) at 946 nm and 976 nm, respectively, using the equations given below. The difference in water estimate ($S_{2es} - S_{0es}$) and the inverse of it ($S_{0es} - S_{2es}$) were plotted in a combined graph (Figure 1) to illustrate the new approach for disease diagnosis.

$$S_{0es} = \text{Free water estimate} = \frac{\text{Absorbance at 946 nm}}{\text{Ref. absorbance at 900 nm}}$$

$$S_{2es} = \text{Bonded water estimate} = \frac{\text{Absorbance at 976 nm}}{\text{Ref. absorbance at 900 nm}}$$

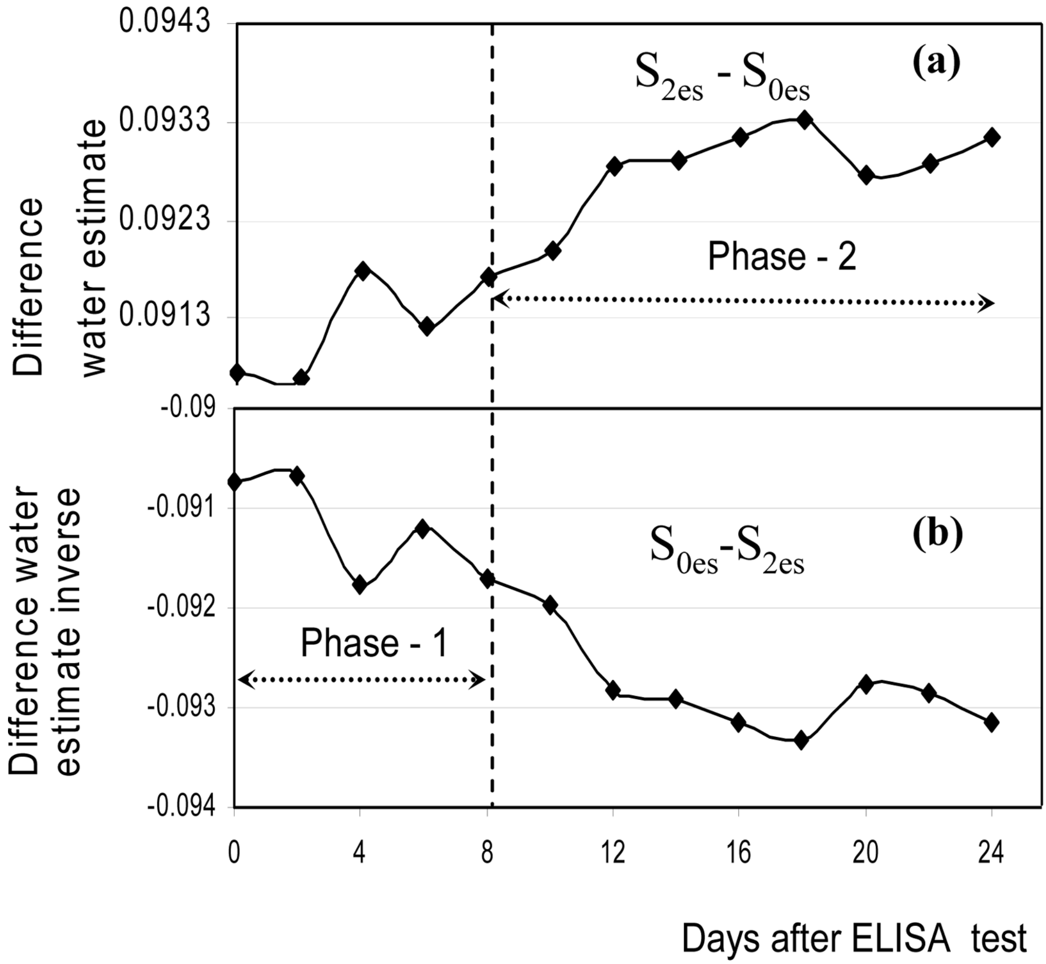

Figure 1. (a) the difference water estimate (S_2–S_0) and (b) the difference water estimate inverse (S_0–S_2).

Table 1. Calculation table of free water and bonded water estimate.

Days	946 nm (S_0 = Free water absorbance)	970 nm (S_2 = bonded Water absorbance)	900 nm (Reference)	Free water estimate 946 nm/ 900 nm (S_0es)	Bonded water estimate 970 nm/ 900 nm (S_2es)	Difference water estimate (S_2es – S_0es)
0	0.6741	0.7249	0.5605	1.2026	1.2934	0.0907
2	0.6945	0.7461	0.5682	1.2223	1.3129	0.0906
4	0.6809	0.7327	0.5651	1.2048	1.2966	0.0917
6	0.6788	0.7296	0.5651	1.2001	1.2913	0.0912
8	0.6737	0.7251	0.5606	1.2018	1.2935	0.0917
10	0.6723	0.7238	0.5599	1.2007	1.2927	0.0919
12	0.6773	0.7289	0.5563	1.2175	1.3103	0.0928
14	0.6811	0.7327	0.5560	1.2247	1.3176	0.0929
16	0.6776	0.7293	0.5547	1.2215	1.3146	0.0931
18	0.6783	0.7302	0.5562	1.2194	1.3127	0.0933
20	0.6798	0.7316	0.5588	1.2164	1.3091	0.0927
22	0.6805	0.7326	0.5604	1.2142	1.3070	0.0928
24	0.6782	0.7302	0.5584	1.2145	1.3076	0.0931

The calculation procedure of these estimates is given in Table 1.

The absorbance values in each column were given in the respective order of day of observation by the average spectrum of each plant category.

Results and discussion

The *SD* profiles of the two plant groups in Figure 2, show that the diseased plants have obtained higher deviation profiles at most of the wavelength positions.

They further reveal that the most sensitive wavelengths towards SMV were those at the observed water bands of non-hydrogen bonded water (S_0) at 946 nm and bonded water (S_2) at 976 nm.[3] In this study, the healthy plant *SD* profile shows a peak at 976 nm. But in the diseased profile this peak was with a 6 nm shift towards the short wavelengths. As Penuelas *et al.*,[4] have reported, this blue shift can be attributed to a shortage of moisture due to the impact of the virus on the diseased plants. Some other studies have described this shift in relation to hydration potential of solutes in water which seems to indicate the existence of different hydrogen bonding activities of water molecules in diseased plants.

Studies based on physical properties of water have reported the occurrence of these structural forms of water under different perturbation.[5] However, observing these water bands from plant leaves as (S_0) and (S_2) with their specific correspondence to plant biology has not been reported. Previous work[4] has demonstrated the ratio of leaf reflectance at 970 nm to the reference wavelength of 900 nm for estimating wild fire risks. In this study, instead of one common water index, we introduce two water indices named "free water estimate" and "bonded water estimate" and the difference of the two named "difference water estimate", to illustrate the water activity in relations to the SMV disease (Table 1).

Time series deviation between the two plant groups

Mean centring and second derivative math transform with six factors of the best model configurations provided the salient information of the disease with its respective two biological phases (Figure 2).

Figure 2 displays a typical scenario of a virus-infected plant.

The graph starts with the statistical distance of 1.1, just after conducting the ELISA test. Then, there was a gradual reduction of statistical distance up to the 8th day, that could be due to the suppression of the disease by protective reactions from the plant. However SMV is a virus disease and the domination of the virulence of the virus can again be seen in the next few consecutive days. As shown in Figure 3, this biological scenario can be divided in to two phases, according to whether the virus or the plant is dominating.

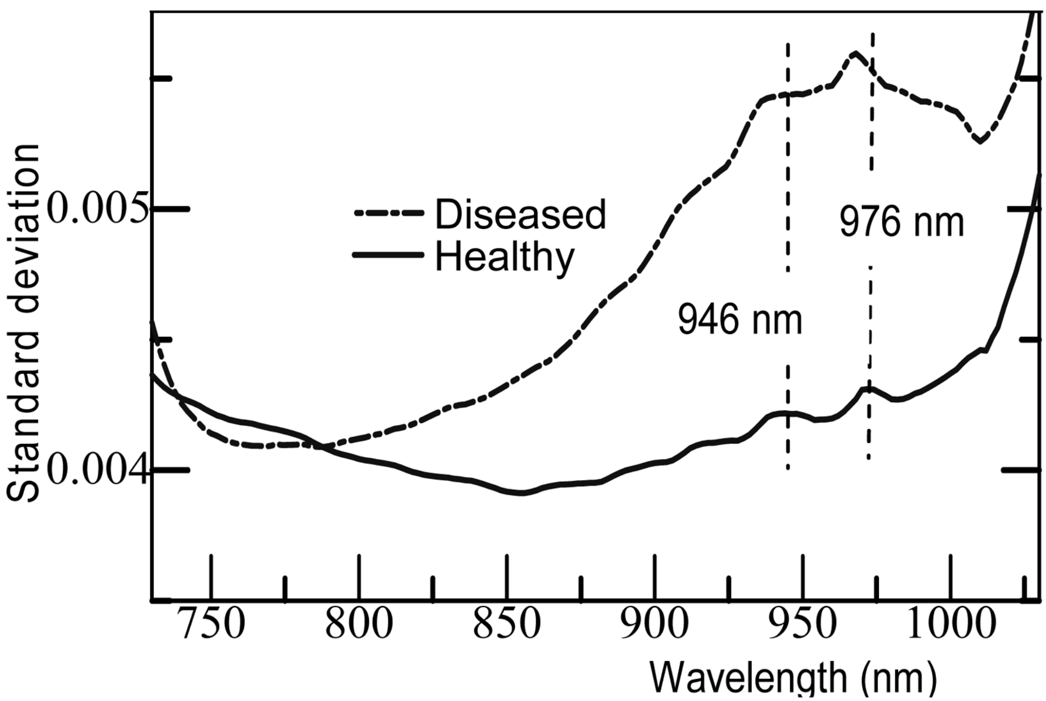

Figure 2. Standard deviation profiles of the two plant categories.

Illustration of SIMCA distance by two water band estimates

The SIMCA results that explain the disease biology in Figure 3 are an output of a multivariate approach that requires all information available at each wavelength of the spectrum. However, using the difference water estimate and its inverse, based on the two water absorbance bands, we could successfully simulate the same biological phenomena.

In phase-2 in Figure 3, the virulence of the virus appeared to be getting higher, showing a continuous increase of class distance. This can be similarly illustrated by the phase-2 of Figure 1(a) by the difference water estimate (S_{2es}–S_{0es}). The phase-1 of the disease depicted by SIMCA distances was in good agreement with the inverse form of (S_{2es}–S_{0es}). When comparing Figures 1 and 3, it is clear that the first phase of the disease was under control of plant vigor, and the increasing severity of the disease was positively correlated (r^2=0.905) to the relative increase of free water. In the second phase, the virulence of virus has strengthened over the plant vigor and there was a negative correlation ($r^2 = -0.79$) with the increasing of free water as the disease progressed.

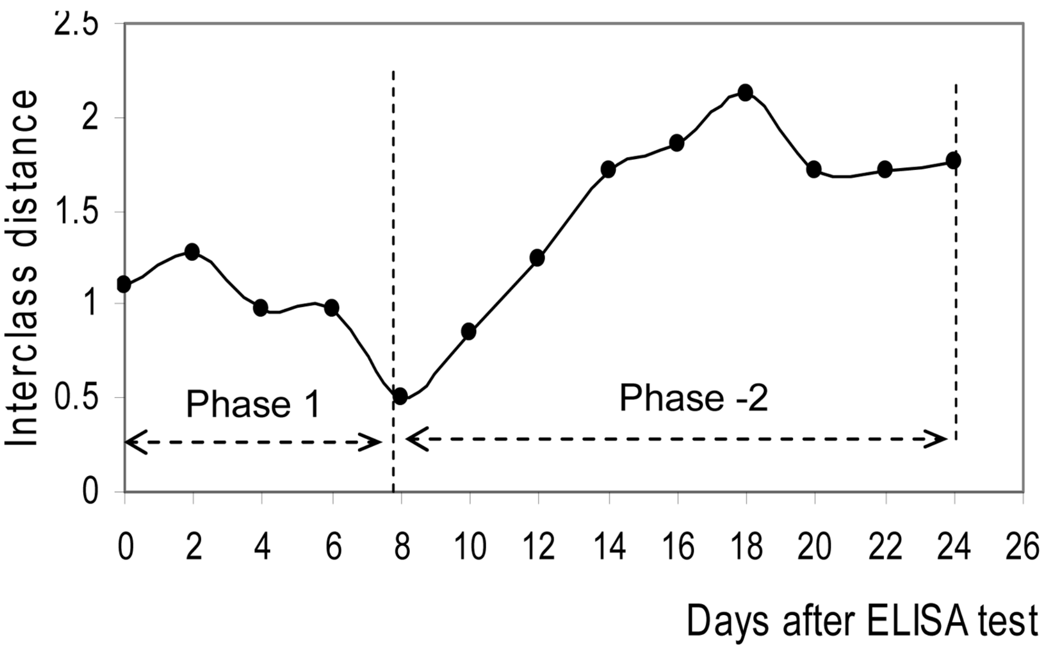

Figure 3. The SIMCA class distance with disease in progress.

Conclusion

Unusual behavior of water structures can be used to follow the progression of SMV disease. Multivariate output was successfully replaced by time resolved analysis of two water bands, showing a new application of Aquaphotomics.

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