ANALYSIS OF PLANT PIGMENTS WITH RAMAN SPECTROSCOPY

APPLICATION NOTE RAMAN-014 (US)

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Abstract

Raman spectroscopy is a valuable tool in detecting environmental stress in plants and can show damage before it is visible to the naked eye.

Motivation and Background

Agriculture is obviously focused on maximizing plant growth and controlling as many of the factors involved in that pursuit as possible. Monitoring the way the environment factors impact the growth of plants, therefore, is of critical interest to the agricultural community, and real-time and non-destructive methods to detect environmental stresses are being constantly developed.

In addition to the many other in-situ and nondestructive analyses possible with Raman spectroscopy, it has also been documented to be useful in phenotyping in plants. In particular, changes in the Raman spectrum can be correlated to changes in the composition of the plants' pigments in response to salinity, drought, cold and light saturation. Environmental stress is a significant problem in agriculture and methods for non-invasively assessing stress damage has been sought on all scales from single leaf to entire farm.

When plants are exposed to abiotic stress, they respond with a series of complex changes involving scavenging of reactive oxygen species. This scavenging is done in part by anthocyanines and carotenoids pigments which are potent antioxidants. Anthocyanine biosynthesis is often caused in the upper epidermis of the leaf by excess light and other abiotic stressors.[1] Changes in the Raman spectra of these compounds, and others, can be detected before the visual appearance of the plant is impacted. In one publication, it is clear that anthocyanines are expressed by the plant following abiotic stress within 48 hours, and that these changes are readily observed with Raman spectroscopy (anthocyanine spectrum monitored from 550-750 cm^{-1}, and carotenoids from 900–1250 cm^{-1}). The features monitored in this publication largely originate in the anthocyanine glycosidic bonds.

Raman spectral changes in the signals related to β-carotene are also readily seen on the 48 hour time scale. This compound rapidly converts to β-cyclocitral in the presence of $^1O_2$. This conversion has been suggested as a molecular signal for the induction of $^1O_2$- responsive genes, therefore is likely one of the major defenses again damage by reactive oxygen.[1,2]

In addition to the spectral signatures of the anthocyanine and carotenoid pigments, chlorophyll is also easily observed in Raman spectra of plant materials. Bands at 744, 1287, 1328 and 1604 cm^{-1} are reported in open literature. Amongst other factors, drought causes chlorophyll loss, predominating in leaf regions, the portion of the plant most subject to water deprivation.[2]

Measurement and Results

A TSI ChemLogix ProRaman-L with 785 nm excitation was used to acquire the spectra of leaves of a common houseplant, variegated philodendron (*Philodendron hederaceum*). This plant is shown in Figure 1. It is clear from a cursory visual inspection that some portions of the leaves are darker green than the others, perhaps because those sections contain less chlorophyll. As previously discussed, many types of abiotic stress on plants cause a diminution of the quantity of chlorophyll in the plant, especially in the leaves, and therefore this type of differentiation could be helpful in establishing and avoiding suboptimal environmental conditions.

Figure 1. A variegated philodendron used for the Raman analyses.

The leaves of the plant were submitted to Raman analysis while still growing on the plant. They were laid flat on an XYZ translation table and covered with a dark veil to prohibit any influence from ambient room light. Efforts were made to keep the laser to sample distance identical for the analysis of each location.

Approximately 100 mW of excitation laser energy was used to analyze two chief regions on a single leaf of the philodendron plant. Each part of the leaf was analyzed for 20s. Signal to noise was improved with averaging and the application of boxcar smoothing (bc-2). The raw signal had an appreciable amount of broadband background associated with it; this baseline was removed with the "autobaseline" function in the RamanReader that is used to run this instrument and prepare the Raman signals for analysis.

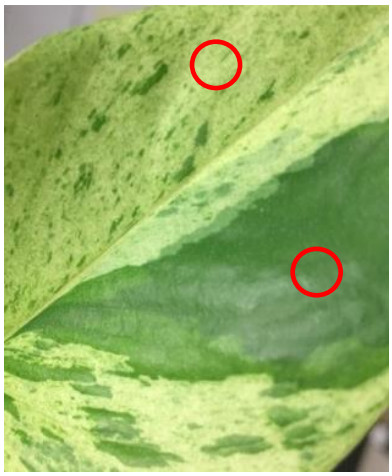

Figure 2. The Raman spectra of the lightest and darkest parts of the leaf are compared below (figure 3).

The two chief regions analyzed with this technique are shown in Figure 2. The circles on this photograph indicate the fact that the spectra below compare the lightest parts of the leaf with the ones that are darkest green; this is not meant to represent the laser spot size, which is much smaller than the circles shown here.

The spectra of both the white and dark green regions are shown in Figure 3. The features designated with arrows, 750, 1292, 1350 and 1554 cm^{-1} originate in the molecular vibrations of chlorophyll .[3] The remaining features can be identified as lignin and glycosidic COC structures.[4]

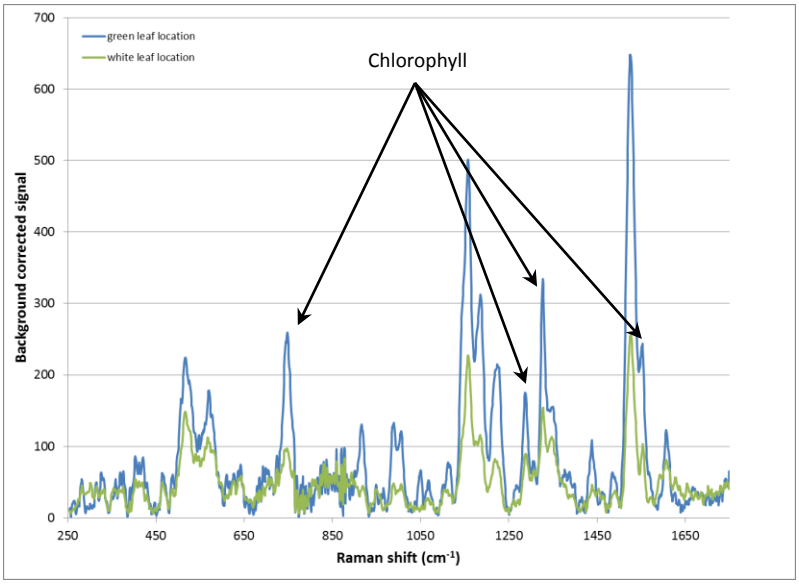

Figure 3. Spectra of both light and dark portions of the philodendron leaf.

It is clear that the lighter regions contain less chlorophyll than the darker green ones; not a surprising conclusion, but rather a confirmation that TSI instrumentation is as capable of detecting these changes as other devices. Another recent application note demonstrates also that the TSI ChemLogix instruments detect lycopene and carotenoid materials from fruits and vegetables.[5] This application note and the previous one about antioxidants demonstrate that the low cost ChemLogix portable instruments have been shown to be ideal for performing this analysis in the lab or in the field.

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