LETTER

Limitations and challenges of using Raman spectroscopy to detect the abiotic plant stress response

Daming Dong[a,1] and Chunjiang Zhao[a,1]

Altangerel et al. (1) suggest that Raman spectroscopy can be used to detect the early abiotic stress response in plants through the measurement of anthocyanins and carotenoids in plant tissues. However, we believe there are some problems that need to be considered.

First, there is insufficient evidence that Raman spectroscopy can be used to measure changes in the composition of plants during the abiotic stress response. The Raman spectral features used in the studies of Altangerel et al. (1) differ from those used in other studies. The most commonly used Raman peak for carotenoid measurement is 1,525 cm^{-1} (2–5), whereas Altangerel et al. (1) used much weaker peaks, at 1,007 and 1,157 cm^{-1}. For the determination of anthocyanins, previous studies have generally used the spectral features between 1,300 and 1,650 cm^{-1} (6). Furthermore, for anthocyanins, the peak at 539 cm^{-1} is stronger than that at 623 cm^{-1} (6), but it was much weaker than the peak at 623 cm^{-1} in the Altangerel et al. (1) study. We note that the intensities of the peaks at 623 and 733 cm^{-1} were nearly equal in figure 3A of ref. 1, but the intensity of the former was much larger than that of the latter in figure 3D of ref. 1. Therefore, we suggest that the Raman shifts measured in the Altangerel et al. study were not only derived from anthocyanins and carotenoids. Changes in compounds other than carotenoids and anthocyanins during the stress response may have disturbed the Raman peaks shown in figure 3 of ref. 1.

Second, there is much room to improve spectra measurements and analysis. First, the measurement scales were quite different among the three methods used in Altangerel et al. (1). The measurement area of Raman microscopy was only 200 μm, whereas the detection scale of the remote spectroscopic system was much larger, although the authors did not give an exact value. In addition, large volumes of plant tissues were used for the traditional chemical analytical extractions, and so this method could not focus only on the epidermis, like Raman spectroscopy. Therefore, it is not suitable to calibrate Raman spectra against data obtained from chemical analytical extractions or to directly compare the three methods, because anthocyanins and carotenoids are not uniformly distributed in plant tissues. Second, we strongly suggest the use of surface-enhanced Raman spectra to enhance the sensitivity of common Raman spectroscopy (6, 7). The spraying of nanoparticles onto the plant surface will not affect on-site measurements in phenotyping.

Finally, the advantages of Raman spectroscopy are overstated. A major drawback of Raman spectroscopy is its poor repeatability, which makes it very difficult to obtain reliable quantitative measurements (8). The problem of poor repeatability is even worse when evaluating the distribution of fluorescence in plant tissues (as indicated by the very large error bars in figure 5 of ref. 1). However, diffuse reflection spectroscopy in the visible, near-infrared, and mid-infrared regions has been used widely in the quantitative measurement of the chemical composition of plant tissues (8), which is as sensitive as common Raman spectroscopy, but with much better repeatability (9). Some portable instruments have been developed based on simple reflection spectroscopy to measure carotenoids in human skin (9, 10).

Acknowledgments

This work was supported by National Natural Science Foundation of China (Grant 31622040).

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[a]Laboratory of Optical Sensing and Physics, National Research Center of Intelligent Equipment for Agriculture, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

Author contributions: D.D. and C.Z. designed research; D.D. and C.Z. performed research; and D.D. wrote the paper.
The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. Email: damingdong@hotmail.com or zhaocj@nercita.org.cn.

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