Title	Use of nondestructive spectroscopy to assess chlorophyll and nitrogen in fresh leaves
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Abstract

Four aspects of factors influencing the accuracy of nondestructive chlorophyll (Chl) and nitrogen (N) measurement in fresh leaves were studied: (1) optimum wavelength (OW) identification; (2) indices development and evaluation; (3) influence of leaf properties; and (4) influence of meter parameters and sampling technique. Results were used to develop indices and prototype meters for Chl and N assessment. Our results indicated that the simple linear coefficient of determination (R^2) between spectral reflectance or transmission and Chl or N in combination with spectral sensitivity was the most reliable method for determining the OW for Chl and N measurement in fresh leaves. There were two ranges of wavelengths, one in visible region (550-580 nm) and the other in the red edge region (700-730 nm), we determined that had the highest spectral sensitivity and largest R^2 with smallest root mean square error over a wide-range of Chl concentrations (160-1188 μmol.m^{-2}), and could be used as the OW to develop indices for Chl and N assessment. The OW in the red edge region could be used for Chl assessment across all species tested and the OW in the visible region could be used across anthocyanin *s*-free species. The best indices were the indices developed with the Chl-related OW either from visible or red edge region in combination with a reference wavelength (RW) from the near infrared (NIR) region (750-1100 nm) that was sensitive to leaf texture but insensitive to Chl as the form of a simple ratio (R_{RW}/R_{OW}) or normalized difference vegetation index ($(R_{RW} - R_{OW})/(R_{RW} + R_{OW})$). With RW, the differences in reflectance in the visible and red edge regions caused by variation in leaf texture or other optical properties could be eliminated. This was particularly important when the R^2 of a single-wavelength index was small for Chl or N measurement (e.g. $R^2 < 0.8000$ for Chl or $R^2 < 0.6000$ for N).

Parameters used by hand-held Chl meters (CCM-200, SPAD-502, and CM-1000) affected their accuracy for Chl and N assessment. Our results showed that SPAD-502 was more accurate than CCM-200 and CM-1000 for assessing Chl and N in fresh leaves. The Chl-sensitive wavelength used by CM-1000 (700 nm) was more accurate for estimating Chl than the wavelengths used by SPAD-502 (650 nm) and CCM-200 (660nm); however, we found that variation in sampling distance, orientation, light intensity, and the inconsistency of light intensity between ambient light sensor and the target leaf made the CM-1000 less accurate than the other two meters. Using the indices and OW determined through our research, we

developed three prototype meters that were more accurate than or similar to the commercial hand-held meters in measuring Chl or N in fresh leaves. Among them, the prototype-III was more accurate than all the commercial hand-held meters for Chl and than the CM-1000 for N assessments across all the species we tested.