Leaf Nitrogen Determination using Handheld Meters

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Abstract

The maximisation of plant nitrogen-use-efficiency (NUE) has direct impact on increasing crop production, due to the increase of agronomic value of the fertiliser. Moreover, optimising the use of nitrogen will reduce the adverse effect on the environment that can be caused by nitrate leaching and nitrous oxide production. Accordingly, nitrogen (N) management in agriculture has been the focus of many researchers. Improvement of NUE can be achieved through measurement of the foliar N content of crops during growth. There are two classes of methods to diagnose foliar N status: destructive and non-destructive.

Destructive methods are expensive and time-consuming as they require tissue sample collection and laboratory analysis. Non-destructive methods are rapid and less expensive, but usually less accurate. Improved accuracy is the goal of many researchers and various methods have been proposed that vary in complexity and optimality. This paper highlights non-destructive handheld methods to estimate foliar N status of plants.

Keywords

Handheld meters, SPAD, LCC, image processing, nitrogen

Introduction

The measurement of foliar nitrogen and chlorophyll contents of plants is very important for agronomic studies of crop growth, yield and quality. Nitrogen is very important to yield because of its key role in cell division, protein synthesis and enzyme production. If cell division is inhibited, leaf area expansion is similarly inhibited and the plant thereby loses its potential to produce a high yield. Despite the importance of N supply to plant growth, excessive supply in fertiliser is costly and excess N that runs off arable land can have a negative impact on the environment. Thus a mismatch between nitrogen supply and crop requirement can hamper crop growth and harm the environment, resulting in low nitrogen-use-efficiency (NUE) and economic losses. There are two classes of techniques for foliar analysis of nitrogen content: destructive and non-destructive. Ramirez (2010) showed that plant nitrogen status can be accurately estimated using a destructive technique; in which foliar samples are analysed using laboratory procedures. This technique is generally time consuming, costly and labour intensive(Sui, Wilkerson, Hart, Wilhelm, & Howard, 2005). In contrast, non-destructive methods can be rapid and less expensive than destructive techniques, but are generally less accurate. There are a number of non-destructive methods available that vary in complexity and optimality. These include use of a Leaf Colour Chart (LCC), which relies on visual comparison between leaf colour and a colour chart to assess the N status of certain plants. One of the most widely used digital tools is the chlorophyll meter (SPAD-502). This is a hand held device that estimates the chlorophyll content of leaves, as leaf chlorophyll content is closely correlated to leaf nitrogen concentration.

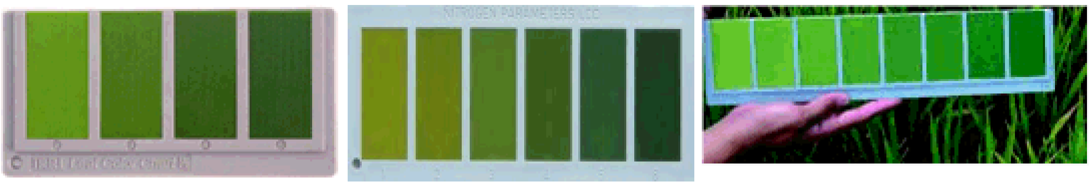
Recently, digital imaging has been applied to plant colour analysis. Digital cameras or scanners in combination with computers and appropriate software can be used to collect images of leaves and evaluate their colour with relative ease and at a reasonable cost. Below is a description of non-digital and digital handheld meters.

Tools for determining the foliar nitrogen content

Non–digital Tool (Leaf Colour Chart-LCC)

Leaf colour is a good indicator for plant health and nutrition. Different types of stress may cause different symptoms and a comparative analysis can yield information about the type of stress. Nitrogen deficient leaves turn to pale or yellowish green rather than dark green and farmers generally prefer to keep leaves of the crop dark green. Figure (1) shows three different types of LCC. Standardised LCC with four colours shade from pale (No.2) to dark green (No.5). Six- panel LCC was an improved version (Figure 1b) (IRRI, 1996; Singh, Singh, Singh, Thind, & Gupta, 2010).Recently, researchers at the University of California developed another eight-panel LCC (UCD-LCC) (Figure 1 c) with scale of eight green colour shades (1–8).

The LCC has mainly been applied to rice crops. Despite the fact that it has made some improvements in NUE for rice growers, the LCC is not an optimal tool to assess foliar nitrogen content because it is affected by a number of factors, such as ambient lighting condition and differences amongst cultivars in leaf colour. Accordingly, the development of a more reliable tool that can accurately detect the onset of N stress before it is visible to the human eye would be valuable, especially if it can be used across a large number of species. Consequently the development of leaf chlorophyll meters has received much commercial interest (Debaeke, Rouet, & Justes, 2006).



1(a) **1(b)** **1(c)**

Figure 1. Leaf Colour Chart (LCC)

Digital Tools

Minolta Co. (Japan) developed the chlorophyll meter (SPAD-502) or SPAD meter (Figure 2). It measures the relative greenness or chlorophyll content of leaves(Turner & Jund, 1994). Because leaf chlorophyll content is closely related to leaf N concentration (Balasubramanian, Morales, Cruz, & Abdulrahman, 1999), this meter has been used to assess foliar N content.

Figure 2. Close-up of SPAD Meter(image taken from http://www.ianr.unl.edu/pubs/soil/g1171.htm)

The SPAD meter estimates the relative chlorophyll concentration in a leaf by measuring the differential transmittance of light through it. Within a small chamber (2 - 3 mm) in which part of a leaf is being held, the meter emits light from two diodes, one producing a peak wavelength near 650 nm (red), which is absorbed by chlorophyll and the other, a peak near 940 nm (infrared), which is transmitted through leaves and serves as an internal reference to compensate for leaf thickness and moisture content(Shapiro, Schepers, Francis, & Shanahan, 2006). More red light is absorbed by leaves when more chlorophyll is present. Thus, the chlorophyll concentrations of leaves are correlated with SPAD meter values. Table (1) shows the comparison between popular handheld instruments.

Table 1. Comparison between popular handheld instruments

Instrument	Wavelength	Measurement Area	Reference
SPAD	650 nm in the red and 940 nm in the near-infrared	12.57 mm2	Netto (2002)
N Tester	650 nm in the red and 960 nm in the near-infrared	2 x 3 mm2	Richardson et al. (2001);Goffart& Olivier (2004) http://bse.unl.edu/adamchuk/gpt_seminar/gpt_present/0510.pdf
CCM-200	660 nm in the red and 940 nm in the near-infrared	0.71 cm²	Richardson et al. (2001)
R meter (CM 1000)	ambient and reflected light at 700 and 840 nm	1.27 cm2	Murdock et al. (1998) Carter and Spiering(1999)

Digital image and colour analysis

An alternative method that is consistent, unbiased, and precise is computer automated digital image analysis (Mirik et al., 2006). Computerised digital image analysis is also a non-destructive and non-invasive method that can capture, process, and analyse information from images. Moreover, this technique allows immediate detection of stressful conditions before visual symptoms appear and adverse effects become established. It is emerging as a promising tool for crop yield management. Furthermore, images are easily archived and stored for future analyses. Colour parameters can be evaluated in different colour systems. The RGB (Red, Green, Blue) colour values have been mostly used to describe and analyse colour images(Erickson, Keziah, Nelson, & Young, 1988).

According to Graeff et al.(2008),when acquiring digital images of broccoli leaves under constant lighting conditions, the image processing technique is faster, more efficient than current techniques and showed a closer correlation with N status than other digital tools. Mercado-Luna et al.(2010) developed a new method to capture images of tomato leaves growing in a green house, where he could control three variations associated with image capture: camera height and camera angle, light levels, and camera settings. He suggested that colour image analysis can be applied to estimate the N status of tomato seedlings using red and blue colours. From colour image analysis, red (R) and blue (B) were the most accurate predictors of N status on plants with R^2 above 0.89. Hence, colour image analysis showed to provide accurate and quick way for N estimation and can contribute to early detection of N deficiency.

Evaluation of LCC, SPAD and image processing-based nitrogen estimation meters

There are a number of factors affecting the suitability and applicability of the different N estimation meters. LCCs are the cheapest, and have been widely used in rice, maize and sugarcane. However, as this approach is based on visual inspection of leaf colour, accuracy is not guaranteed, especially for different lighting conditions. SPAD, on the other hand, is less sensitive to lighting conditions, but has not shown consistent performance across all species. The fluctuation of SPAD performance is influenced by its small measuring area (around 12.57mm^2). The relatively high cost of SPAD makes it less appealing for small farmers, especially from developing countries. Similar to LCC, image processing techniques are also affected by the environmental conditions as these have to be set appropriately to produce reliable results across different species. Despite being only applied to limited number of species, image processing showed good potential compared to SPAD and LCC. Image processing technique may require calibration, but its cost is generally less than that of SPAD. In general, each of these methods may have their own advantages and disadvantages. However, the image processing technique is yet to reach its full potential. Table 2 shows a comparison between LCC, SPAD and image processing based nitrogen estimators.

Table 2. Effect of different factors on LCC, SPAD and image processing-based nitrogen estimators.

Factors	LCC	SPAD	Image processing technique	Reference
Applicability to wide range of species	More relevant for cereals grasses?such as rice, maize and sugarcane ?????	Has been applied to a number of species, but did not produce accurate results for others. For example, not suitable for leaves of regenerated plants.	As this is a relatively new technique for N estimation, it has been applied to limited number of species. However, it has the potential to be applied to a wide range of species.	Nagappa et al. (2002);Yadav et al.(2010)
Effect of environment on readings	As direct sunlight affects leaf colour readings, it is recommended to take the reading in the shade.	Environment has no or very little effect on readings.	Similar to LCC, sunlight has an effect on the colour of the acquired image.	IRRI (1996)
Accuracy	Accuracy is not guaranteed, as reading depend on visual assessment.	Can be quite accurate. However, due to the small measuring area of the device (around 12.57 mm2), the accuracy is limited	Has the potential to produce accurate reading when images are collected in optimal environment/setting.	IRRI (1996); (Netto, et al., 2002)
Potential for improvement	Limited improvement only, through increasing the number of panels (green shades)	Improvement in the underlying technology. The latest version is SPAD 502	Has the potential for further improvement by enhancing the image processing technique.	IRRI (1996);Yang et al. 2003); Yang et al. (2003); Uddling et al. (2007)
Calibration	Does not require calibration	Does not require calibration	Digital cameras may require calibration	
Price	USD $1	USD $2200	Depends on the type of camera, but costs usually lower than SPAD	Balasubramanian et al. (2000)

Conclusions

Handheld meters for N detection provide rapid and computationally efficient solutions. However, these methods vary in their optimality, cost and applicability. Digital tools based on chlorophyll measurements (for example, the SPAD) can produce better results than non-digital ones, such as

LCC. However, they are far more expensive. On the other hand, SPAD reading is not very consistent across all plants.

Image processing methods have started to attract increasing attention, as they produce encouraging results. However, there is a continuous need to enhance their accuracy by controlling several factors including: constant light, constant height and angle, constant camera setting (manual or automatic) and robust software algorithms for processing and analysing leaf images. Control of these factors can contribute to early detection of N deficiency and hence, lead to N application at the correct amount at the right time.

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