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Use of a digital camera as alternative method for non-destructive detection of the leaf chlorophyll content and the nitrogen nutrition status in wheat

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ABSTRACT

In this paper, the use of a digital consumer camera for the non-destructive detection of the *N* nutritional status is compared with two alternative methods, namely SPAD and reflectance spectrometry in three field experiments. The image analysis method consisted of segmentation and successive analysis of the foreground color, i.e. only green plant parts. Thus, also analysis of canopies with small degree of ground cover is possible. All methods gave comparable results, while the effort necessary was considerably higher when using the chlorophyll meter. With spectral measurements, the biomass and leaf nitrogen content could not be clearly differentiated; chlorophyll measurements do not reflect biomass, whereas the described procedure of image analysis permits the consideration both. If used properly, digital image analysis is a valuable tool for the determination of the *N* nutrition status under field conditions, with low costs and labor requirements.

1. Introduction

A repeated assessment of the leaf nitrogen content are often necessary in field experiments, but destructive determination is often limited by the size of the plots and efforts required for sampling. Moreover, errors resulting from small sampling areas are often high. Non-destructive measurements may be applied frequently and repeatedly on the same area, if necessary also on the whole plot and may be preferred, if absolute values of the nitrogen content or uptake are not required.

Possible methods for the non-destructive measurement of the leaf chlorophyll content as follows: (i) spectrometric measurements using spectral indices such as the normalized differential vegetation index (NDVI) or the red edge inflection point (REIP) (Erdle et al., 2011), (ii) use of the SPAD chlorophyll meter or similar devices (e.g. the atLEAF Chlorphyll meter, FT Green LLC, Wilmington, DE, USA) (Yue et al., 2015) and (iii) digital image analysis, in which the color of leaves or other mgreen plant parts is analyzed. (Moghaddam et al., 2010; Pagola et al., 2009; Vollmann et al., 2011; Wiwart et al., 2009). Spectrometers for field measurements are not always available, are rather expensive and perform integral measurements, without spacial differentiation within the field of view. The SPAD enables a rather accurate measurement of the chlorophyll content, but not of the ground cover or the fraction of biomass; however, its use is rather laborious. Digital cameras are now commonly available; digital image analysis has the potential to independently combine the direct determination of soil ground cover (and thus, with some limitation to estimate biomass and the LAI), and the leaf chlorophyll content.

Numerous studies have reported the use of digital image analysis to determine the nutrition status, chlorophyll content or other criteria based on leaf color, such as the differentiation among species or nutrient deficiencies other than *N* (Moghaddam et al., 2010; Pagola et al., 2009; Vollmann et al., 2011; Wiwart et al., 2009). However, detailed descriptions of the methodology are often lacking (limiting method descriptions often with regard to the names of software packages used). Most use detached leaves under controlled conditions. Field measurements are frequently performed on the whole photographed area (i.e. including soil), thus neglecting the main advantage of image analysis, namely

Abbreviations: ExG, green excess index; LAI, Leaf Area Index; ME, misclassification error; NDI, normalised difference index; NDVI, normalised difference vegetation error; NIR, near-infrared; PLSR, partial least squares regression; REIP, red edge inflection point.

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the possibility of excluding pixels belonging to soil or other background from the analysis.

Assessments of the efficiency of the methods are mostly limited to correlations with destructively determined parameters. This demonstrates basically the potential of the methods, but provides little information on the accuracy of the assessments performed. Here, more detailed analyses with respect to optimum sampling areas and the minimum number of measurements are necessary to decide, which method to apply and for planning of experiments.

The Aim of this work is as follows: (1) to describe suitable methods of image analysis, that are easy to implement and use, without referring to determinate software packages (2) to compare their ability to estimate the chlorophyll or leaf nitrogen content and (3) to provide measures of accuracy, both for a comparison of methods and as guiding values for planning.

Two basic procedures for image analysis are of particular importance, *segmentation* and *color analysis*.

Segmentation is the first step in most protocols of digital image analysis. The aim here is, to convert the color image into a binary image (segmentation), that subdivides the image into a foreground (green plant parts) and background, (mostly soil and dead plant parts). Thus, the pixels belonging to the soil or other background may be identified and can be excluded from the analysis.

Almost all authors achieve this with color analysis. Only in a few cases, however, particularly if crops and weeds have to be differentiated also texture analysis or object-orientated approaches have been used (Cointault and Gouton, 2007; Gebhardt and Kuehbauch, 2006; Gebhardt et al., 2006; Laliberte et al., 2007). However, the sole differentiation of green plant parts and soil is not a major challenge and, as has been shown by the authors cited above, can be achieved by relatively simple procedures of color analysis.

Commonly, digital images are saved as 24 bit RGB images, where spectral information associated with the basic colors of red, green and blue are distributed in separate layers and each pixel is associated with a vector of three elements, indicating the intensities of the color bands, red (R), blue (B) and green (G), which range between 0 and 255.

The first step is, with to use appropriate arithmetic procedures, to convert for each pixel of the intensities of the three color bands intensities to into a unique index, where the values of the foreground -and background- pixels are possibly distant from each other, allowing in a second step, the separation of fractions by simple thresholding in a second step. In plant parts appearing green to the human eye, G predominates the other two bands (R and B); therefore, the index should express the degree of this predominance.

Such indices may be based on *ratios* or *normalized differences*. Using *simple ratios*, such as G/R, $G/(R+B)$, and so on, the relationship with the chlorophyll content or related parameters may not be linear and extremely high or low values as well as frequent divisions by 0 may occur. Thus, most indices represent normalized difference indices, which are limited to the range from −1 to +1. Normalized indices may be based on 2 or three color channels; the "normalized difference index" of Woebbecke et al. (1995) (*NDI*) is the most frequently used measurement of plant canopies and describes the green/red ratio:

$$NDI_{gr} = \frac{G-R}{G+R} \tag{1}$$

The NDI can be extended including to also include the blue obtaining the "Green excess index" (*ExG*), which was also introduced by Woebbecke et al. (1995):

$$ExG = \frac{2G-(R+B)}{R+G+B} \tag{2}$$

Both indices have been demonstrated to be valuable for the distinction of green plant parts and soil (Woebbecke et al., 1995).

Numerous automatic procedures for the identification of threshold values have been described; 40 of them were compared and discussed in a survey by Sezgin and Sankur (2004). However, only a few methods have been applied to agricultural research. We tested several automatic thresholding procedures (unpublished data), and found, that the use of a fixed threshold, identified manually for a set of images taken under similar light conditions is more reliable and accurate, and does not require interactive control of each image.

Color analysis is aimed at characterizing integral images or segments in a ratio of the three basic colors, i.e. red, green and blue. It has been performed with different aims such as assessments of diseases, stress symptoms or senescence in general (Bock et al., 2010; Diaz-Lago et al., 2003; Douches et al., 2002; Kipp et al., 2014b), distinction between different plant species (Himstedt et al., 2009; Sokefeld et al., 2002; Bonesmo et al., 2004; Chen et al., 2010), assessment of biomass (Jensen et al., 2007; Kipp et al., 2014a) and of the *N* nutrition status (Jensen et al., 2007; Moghaddam et al., 2010; Vollmann et al., 2011) or deficiency of nutrients other than *N* (Wiwart et al., 2009). Color analysis is performed in different ways: some authors use photographs from homogeneous areas of detached leaves or other plant parts photographed under controlled conditions (Wiwart et al., 2009; Vollmann et al., 2011), others analyze the average color of the whole area of the image of a canopy, mostly in the field (Casadesus et al., 2007; Himstedt et al., 2009; Jensen et al., 2007; Moghaddam et al., 2010), but in only a small part of these reports, the main virtue of image analysis, namely the possibility to perform first a segmentation and color analysis in a second step on determined segments has been used (Sokefeld et al., 2002). Of particular interest is the possibility to assess the *N* nutrition status based on the leaf color (Moghaddam et al., 2010; Pagola et al., 2009). Vollmann et al. (2011) compared several color indices with measurements performed with the SPAD chlorophyll meter and found high correlations. However, all of these studies were either performed on images of single leaves under controlled conditions (Pagola et al., 2009; Vollmann et al., 2011), or the analysis was performed integrally, without previous segmentation. The color indices are basically those used also for segmentation: simple ratios and normalized difference indices. A few authors use parameters of the HSV color system, but there is no evidence, that these are more suitable.

2. Materials and methods

2.1. Field experiments

We analyzed datasets originating from three field experiments conducted in 2010/11 and 2012/13 at the Dürnast research station of the Technical University of Munich in Bavaria, Germany (11°41′60″E, 48°23′60″N). The soil is characterized as a mostly homogeneous cambisol of silty clay loam with a water holding capacity of about 160 mm within a soil depth of 1 m. The fields are located in a hilly region sloping slightly northwards. at approximately 0.09 m m^{-1}. The average yearly precipitation in this region is 787 mm, and the average temperature is 7.8 °C (data measured by "Deutscher Wetterdienst", 1971–2000). We analyzed data sets derived from different experiments performed in 2010/11 and 2012/13.

2.1.1. Experiment 1

The bi-factorial experiment was established in the fall of 2010. Four levels of nitrogen fertilization and 7 German commercial bread wheat varieties were compared. The design