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Assessment of plant nitrogen status using chlorophyll fluorescence parameters of the upper leaves in winter wheat

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ABSTRACT

Non-destructive, rapid diagnosis of plant nitrogen status is important for the evaluation of wheat growth and the dynamic management of nitrogen nutrition. Two wheat cultivars, Zhengmai 366 (high protein content) and Aikang 58 (medium protein content) were grown in field trials at five different nitrogen levels (0, 90, 180, 270 and 360 kg ha^{-1}) in two consecutive growing seasons at Zhengzhou, China. Leaf chlorophyll fluorescence (ChlF) parameters, leaf and stem biomass, and nitrogen content were measured simultaneously at different growth stages, establishing an evaluation model of plant nitrogen nutrition in wheat using ChlF parameters. The results showed that the differences in ChlF parameters between the three top leaves (1–3LFT) was small from the reviving to the flowering stages. With increasing nitrogen levels, the difference in ChlF parameters between the fourth leaf (4LFT) and the first three leaves (1–3LFT) decreased gradually, indicating that 4LFT is sensitive to N fertilizer application and has a disadvantage in competition for redistributed N. The correlation coefficients between ChlF parameters for the upper, fully expanded leaves and N concentration of the corresponding leaves were 0.628 for F_v, 0.607 for F_m, 0.579 for F_v/F_o, and 0.600 for F_v/F_m at $P<0.01$, but only 0.248 for F_o at $P<0.05$. At the reviving and jointing stages, the relationships between the normalized differences between 1–2LFT and 4LFT ($NDF_{12/4}$) for F_v/F_o and F_v/F_m to plant nitrogen concentration (PNC) were the most significant ($r<-0.79$, $P<0.001$), the determination coefficient (R^2) for F_v/F_m was much higher than for F_v/F_o, and the two regression equations were grouped at reviving and jointing with similar R^2 values between the stages. At booting and flowering, the normalized differences between 1–2LFT and 4LFT for F_o, F_m, and F_v better reflected the changes in PNC; the R^2 values were 0.654–0.797 ($P<0.001$) at booting and 0.515–0.584 ($P<0.001$) at anthesis, and the two regression equations were grouped at booting and anthesis with greater differences in R^2 between the stages. The unified regression equation could be used to express the relationship between plant nitrogen sufficiency index (NSI) and ChlF parameters with R^2 values of 0.623 ($P<0.001$) for $NDF_{12/4}$ for F_v/F_m, and 0.567 ($P<0.001$) for $NDF_{12/4}$ for F_v/F_o during the reviving and jointing stages, while $R^2=0.666$ ($P<0.001$) for $NDF_{12/4}$ for F_m and 0.615 ($P<0.001$) for $NDF_{12/4}$ for F_v during booting and anthesis. These results show that the relationship between NDF and NSI was stable and reliable over the different years, varieties, and N supply levels. We conclude that the spatial differences in ChlF parameters between 1–2LFT and 4LFT should be ideal indicators of plant nitrogen status in wheat, and will provide a decision-making method for N diagnosis and regulation in field production.

1. Introduction

Nitrogen (N) is one of the essential nutrients for growth, yield, and quality in wheat. Although the per-hectare yield of wheat increases to some extent when nitrogen fertilizer is increased during the growing period, nitrogen utilization is significantly reduced, which can result in increased environmental pollution (Zhao et al., 2006). Excessive nitrogen fertilization may cause plant lodging, undesirable delayed senescence at later stages of growth,

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an increase in the incidence of pests and diseases, and deterioration of grain quality (Subedi et al., 2007; Drinkwater and Snapp, 2007). Crop growth and nitrogen status need to be evaluated early in order to rationally plan nitrogen management, to allow timely diagnosis of nitrogen and regulation of fertilizer applications. Conventional nitrogen management relies on a combination of morphological diagnosis of field crops and indoor test analysis; this not only requires a considerable investment in manpower and material resources for sampling, measurement, and data analysis, but is also an inefficient use of time. Fast, non-destructive, accurate monitoring and diagnosis of wheat nitrogen nutritional status will help to determine the best management strategy and dynamic regulation of nitrogen use in wheat.

Recently, the use of non-destructive plant phenotyping technology to supplement soil testing has attracted attention in the diagnosis of crop nitrogen nutrition and for recommending nitrogenous fertilizer applications. Several research groups are developing various sensor systems for field applications, and these devices are characterized by different excitation wavelengths and active/passive capturing modes. Recently, a SPAD meter has been applied to wheat, corn, grass, and other crops to evaluate crop nitrogen status (Duru, 2002; Debaeke et al., 2006; Noura, 2008), but the relationships are vulnerable to genotypic characteristics, different production methods, and environmental effects. The appropriate SPAD values were estabished on the basis of their relationship to crop yield under high-yield conditions (Prost and Jeuroy, 2007; Hawkins et al., 2007; Ziadi et al., 2008), but they are usually suitable for similar crop varieties, growth stages, and growing region. Debaeke et al. (2006) used the relative SPAD value of the first leaf from the top in wheat (RSPAD), instead of the original SPAD value to build a relationship with nitrogen nutrition index (NNI). RSPAD could indicate changes in NNI by eliminating the influence of the environment. However, computing the RSPAD values required high nitrogen treatments as a control, which had some limitations in the practical application. Alternatively, the use of the digital camera as a low-cost, efficient tool has been examined in recent studies. Digital camera images provide spectral information in the red, green, and blue (RGB) wavelength to reflect the properties of crop or background surfaces (Lailiberte et al., 2007). Image analysis of color digital images has been used successfully in the evalation of crop growth and N nutrition status only during the early vegetative stages in wheat and rice (Li et al., 2010; Lee and Lee, 2013), but the estimation error of plant N content was found to sharply increase due to architectural changes at higher levels of canopy cover and above-ground biomass.

Recently, hyperspectral remote sensing technology has been shown to be a promising tool to rapidly monitor crop growth status and assess the spatial variability of a crop field over a large area (Hansen and Schjoerring, 2003; Ecarnot et al., 2013). Plant N concentration can also be estimated with nondestructive remote sensing techniques, since chlorophyll content is closely linked to nitrogen status (Martin and Aber, 1997). A number of research groups have demonstrated the potential of hyperspectral remote sensing for assessment of plant N content using different analysis techniques, such as single band reflectance, two-band vegetation indices, three-band vegetation indices, multiple linear regression (SMLR), and partial least squares (PLS) (Feng et al., 2014; Li et al., 2012; Ecarnot et al., 2013). However, monitoring methods based on reflectance have the drawback of providing a mixed measurement signal originating from both the plants and the soil. Moreover, the accuracy and robustness of these different methods may be inadequate to guide producers in managing N application during the critical growth stages at both field and regional scales, especially under intensive, high yield cultivation conditions.

Chlorophyll fluorescence (ChlF) is a reaction of the photosynthesis apparatus in which excess light energy that is not consumed in the photochemical reactions or converted to heat is radiated from the plant as fluorescent light. As an active remote sensing technology, ChlF detection has been successfully used to monitor the health and growth of plants. ChlF measurement provides a promising new perspective as a sensor solution. A major advantage of such a method is that fluorescence signals originate only from the plants. In stress physiology, ChlF is an important, non-invasive technique used to assess and quantify damage to the leaf photosynthetic apparatus, particularly PSII activity, in response to environmental stresses such as heat, drought, salinity, nutrition, and graft stress, and ChlF has been shown to be a reliable indicator of plant stress (Baker and Rosenqvist, 2004; Sharma et al., 2014). Faraloni et al. (2011) found that the decrease in the F_v/F_m ratio was closely related to changes in the morphometric and anatomical parameters measured in olive under drought stress. There were close correlations between various ChlF parameters (F_v/F_m, photosystem II quantum yield, etc.,) and quality-related properties in vegetables (Toivonen and DeEll, 2001; Bengtsson et al., 2006). ChlF parameters were shown to be significantly affected by nutrient element supply, with increases in non-photochemical quenching (NPQ) and fast-relaxing NPQ (NPQ_F) in rice as N levels increased (Shrestha et al., 2012), and decreases in Fv/Fm, ΦPSII, ETR, and qP at excessive N fertilizer levels in cotton (Liu et al., 2008), and also for RFd under excess Zn levels in tomato (Cherif et al., 2010). Due to the ease of use and non-invasive nature of ChlF measurement, some key ChlF parameters of dark-adapted leaves can be used as a high-throughput screening tool for monitoring temperature stress in crops (Baker, 2008; Janka et al., 2013; Sharma et al., 2014), and growth of phalaenopsis seedlings (Hsu, 2007) by F_v/F_m, and for evaluating genotypes for salt tolerance using qp, NPQ, and ETR (Zribi et al., 2009). Presently, there have been few report on the spatial variation of ChlF in the upper leaves of wheat and the quantitative relationship with plant N status. In the north China plain area, winter wheat is typically fertilized twice with N, once prior to seeding and a second application as topdressing at certain specific stages from reviving to heading. Reduced canopy cover could lead to a higher proportion of soil area during earlier vegetative stages such as reviving and jointing, and mixed reflectance signals from both the plants and soil at canopy levels seriously affects the stability and accuracy of correlations between plant N status and reflectance characteristics. In this paper, we propose the use of ChlF as an alternative method to evaluate whole-plant N status to effectively implement site-specific fertilization regimes.

The primary aim of this work was to evaluate the suitability of ChlF to accurately determine plant N status in winter wheat using a hand-held device under field conditions. Based on field trials with different wheat varieties at different N levels, a portable ChlF analyzer was used to conduct non-destructive measurement of fluorescence parameters at different leaf positions in winter wheat, to construct normalized differences in ChlF between the fully expanded leaves at the top in order to develop an evaluation model of plant N status. The anticipated results will provide technical support and a theoretical basis for diagnosing N nutrition and for recommending fertilization of the wheat crop using the ChlF technique.

2. Materials and methods

2.1. Experimental design

A completely randomized block design with two-way factorial arrangement was used for two winter wheat cultivars with four N treatments replicated three times. This experiment was conducted at the experiment station of Henan Agricultural University located in Zhengzhou in 2008–10 growing seasons. Two types of wheat