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Evaluating canopy spectral reflectance vegetation indices to estimate nitrogen use traits in hard winter wheat

Katherine Frels[a,*,1], Mary Guttieri[a,2], Brian Joyce[b], Bryan Leavitt[c], P. Stephen Baenziger[a]

[a] Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583-0915, United States
[b] Independent Researcher, Farmington, MN 55024-8088, United States
[c] CALMIT, University of Nebraska, Lincoln, NE 68583-0915, United States

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ABSTRACT

Wheat nitrogen use efficiency must be improved to reduce the need for nitrogen (N) fertilizers. This study was conducted to determine if measurement of canopy spectral reflectance (CSR) could be used to non-destructively and indirectly select wheat genotypes with improved N use traits. Canopy spectral reflectance measurements were collected during grain fill in a 299-genotype trial planted near Ithaca, NE in 2012 and 2013, and 28 vegetation indices were calculated from the data. The relationship between vegetation indices (VIs) and nitrogen use (NU) traits was investigated. Vegetation indices were highly heritable in both years and predicted these NU traits: anthesis biomass, mature biomass, grain N yield, grain yield, N harvest index, N utilization efficiency, N uptake efficiency and post anthesis N uptake. One VI, Maccioni, performed most consistently and was significantly related to several NU traits. The results of this study indicate that VIs, particularly the Maccioni index, could be used in wheat breeding programs to non-destructively phenotype for NU traits.

1. Introduction

Wheat (*Triticum aestivum* L.) is fertilized with inorganic N to maximize grain and forage yields and to increase grain protein concentration. In the US alone, 1.5 MMT of N were applied to wheat in 2011 (Nehring, 2013). However, it is estimated that 50–70% of the N applied to the soil is not taken up by the plant (Masclaux-Daubresse et al., 2010). This nitrogen can be leached from the soil and contaminate surface and ground water leading to harmful effects on human health (Wolfe and Patz, 2002), algal blooms and hypoxia in coastal waters (Rabalais et al., 2001), and denitrification that results in greenhouse gas emissions (Beaulieu et al., 2010).

Nitrogen use efficient wheat genotypes would maintain current yield levels with lower N fertilization, or have higher yield with the same level of N fertilization as current practices (Ortiz-Monasterio et al., 2001). Thus, nitrogen use efficient genotypes can reduce N pollution (Hirel et al., 2007). Moll et al. (1982) defined N use efficiency (NUE) as grain dry matter yield per unit N available from soil or fertilizer, which can then be separated into two separate processes: the efficiency with which N is taken up from the soil, or N uptake efficiency (NUpE), and the efficiency with which N in the plant is remobilized to produce grain, or N utilization efficiency (NUtE). High NUpE wheat genotypes efficiently absorb N from the rhizosphere and reduce the amount of N available in the soil for leaching, while high NUtE genotypes efficiently convert the N in the plant into grain yield.

Nitrogen use efficiency evaluation in hard wheat used for bread making must also consider the relationship between N and grain protein content. Nitrogen remobilization efficiency is important to wheat, as N remobilized from the plant to the grain increases the grain protein content. Genotypes with high remobilization efficiency transport 50–90% of the N from the plant biomass to the grain (Kichey et al., 2007), leading to higher protein content in grain. Nitrogen harvest index (NHI) is defined by Sinclair, 1998 as N yield in grain per total aboveground N and reflects the effect of N remobilization on grain protein content. Post anthesis nitrogen uptake (PANU) is an estimate of the amount of N taken up by the plant after flowering (Foulkes et al., 2009). Grain N content is strongly correlated with PANU (Kichey et al., 2007), suggesting that grain protein content can be improved or maintained by selecting wheat genotypes that continue to take up N after flowering. Many studies have found genetic variation for these physiological NU traits, which suggests that NUE can be improved through breeding for NU traits (Foulkes et al., 2009; Guttieri et al.,

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[*] Corresponding author.
E-mail address: katherine.frels@gmail.com (K. Frels).
[1] Present address: Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108-6026, United States.
[2] Present address: USDA Agricultural Research Service, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506-5502, United States.

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2017; Hirel et al., 2007).

Although genetic variation for NU traits is available and the need for improved NU is well documented, active selection for NU traits is uncommon. Phenotyping NU traits is labor intensive, time consuming, and requires destructive biomass harvest. For example, to estimate PANU, two biomass harvests are required, one at flowering to estimate N yield at anthesis (ANY) and another at maturity to estimate N yield in both mature vegetative tissues and grain (GNY). Due to the labor and time requirements that must be invested in the biomass harvest and the destruction of experimental plots, few breeding programs empirically estimate NU traits during selection (Aparicio et al., 2000; Feng et al., 2008; Foley et al., 1998), which greatly restricts the ability to develop genotypes with improved NUE. Therefore, alternative methods that are less expensive and less labor intensive are needed to rapidly assess many wheat genotypes for NU traits.

Proximal sensing of the canopy, measuring CSR, is a promising alternative phenotyping method (Araus and Cairns, 2014). Canopy spectral reflectance is high-throughput, non-destructive, repeatable, and can be collected in a short time period with relatively low labor requirements (Cobb et al., 2013; Tuberosa, 2012; White et al., 2012). Previous studies have used CSR used to detect N deficiency in maize (Bausch and Duke, 1996; Sripada et al., 2008), wheat (Rodriguez et al., 2006) and rice (*Oryza sativa* L.; Takebe et al., 1990). These studies evaluated N stress in the crops by estimating leaf or canopy chlorophyll content using proximal sensing. Other studies have predicted grain yield using CSR estimation of chlorophyll content (Aparicio et al., 2000; Babar et al., 2006; Bowman et al., 2015). Estimates of leaf chlorophyll are highly correlated with estimates of leaf N concentration (Feng et al., 2008; Peñuelas et al., 1994; Yoder and Pettigrew-Crosby, 1995). Therefore, CSR could be used to estimate N content and NU traits in the plant (Pavuluri et al., 2015).

Vegetation indices (VIs) are mathematical calculations of canopy reflectance at specific visible and near-infrared wavelengths. These indices were developed to estimate changes in canopy chlorophyll content and thereby to predict plant N status (Gutierrez-Rodriguez et al., 2004). Many different VI have been used to estimate canopy chlorophyll, but there is little agreement on which VI are superior for detecting chlorophyll in wheat (Haboudane et al., 2002; Main et al., 2011). Main et al. (2011) investigated the relationship of 73 vegetation indices with total chlorophyll content and ranked indices based on performance in multiple plant species. They found that indices using the red-edge region (680–730 nm) were robust across species and predicted canopy chlorophyll content with greater linearity and less saturation, better than other VIs.

Although CSR has been used in precision agriculture for the last 15 years, it is a relatively new tool for phenotyping in breeding programs. Proximal sensing strategies developed in precision agriculture studies can be adapted for phenotyping experimental lines (Gutierrez-Rodriguez et al., 2004). For example, Feng et al. (2008) used proximal sensing to monitor leaf N concentration during grain filling and found a strong relationship ($R^2 > 0.80$) between several VI and leaf N content at specific growth stages. However only six wheat cultivars were tested in plot sizes of up to 27.5 m^2. Testing only a few genotypes in large plots is not realistic for plant breeding programs. Depending on the generation, a breeder may test hundreds to thousands of genotypes in small ($< 5\ m^2$), unreplicated plots. Therefore, if CSR is to be useful by plant breeders for phenotyping NU traits, VI relationships with NU phenotypes must be investigated on the scope and scale relevant to a breeding program.

The first objective of this paper is to determine the VI most useful for accurately estimating NU traits in the context of a winter wheat breeding program. Many VIs have been developed to estimate chlorophyll content but have either not been evaluated in winter wheat or have been evaluated with only a few genotypes in large plots. This study evaluated 28 VIs to test the relationships with NU traits (NUpE, NUtE, NHI, PANU, GNY) and the general agronomic traits (biomass at anthesis (AB), biomass at maturity (MB), and grain yield (GY)). The second objective is to determine the most predictive stage(s) of the grain fill period between heading and physiological maturity for CSR phenotyping for NU trait prediction. Results from this study can be used to develop a simpler, high throughput phenotyping protocol that is time and labor efficient for NU trait selection in hard winter wheat and related cereal crops.

2. Materials and methods

2.1. Plant materials

A panel of 299 elite hard winter wheat genotypes from the Great Plains region of the United States (Guttieri et al., 2017, 2015) was evaluated for NU traits and standard agronomic traits. The genotypes chosen represent cultivars widely grown in the region, those that have contributed significant genetics to current breeding lines, and advanced experimental lines that may be released in the future. The panel contained 193 cultivars and 106 advanced breeding lines ranging from historical cultivars developed prior to 1960 to releases as recent as 2014. The genotypes were submitted by breeders from both public and private breeding programs in South Dakota, Montana, Nebraska, Kansas, Colorado, Oklahoma, and Texas.

2.2. Experimental design

The trial was grown in 2012 and 2013 near Ithaca, Nebraska. The soil is classified as a Yutan series fine-silt, mixed, superactive, mesic Mollic Hapludalfs (Survey, 2007). Soil characteristics were measured from samples taken throughout the field in early spring in both 2012 and 2013 (Supplemental Information A). Seed treatments were applied prior to planting and fungicides were applied after heading as described in Guttieri et al. (2015) to avoid the confounding effects of insect and disease damage on NU traits.

The trial was conducted as a split plot arrangement of an augmented design. Two nitrogen treatments, moderate and low, were arranged as whole plot treatments with two replications of each nitrogen treatment. Nitrogen treatments were designed based on yield goals of 4000 $kg\ ha^{-1}$ in 2012 and 4700 $kg\ ha^{-1}$ in 2013. Moderate N treatments had 120 $kg\ ha^{-1}$ and 126 $kg\ ha^{-1}$ residual + applied N in 2012 and 2013 respectively. Low N treatments had 75 $kg\ ha^{-1}$ residual + applied N in 2012, while in 2013 the low N treatments received no additional N for a total of 82 $kg\ ha^{-1}$ residual soil N. Genotypes were arranged as subplot treatments in plots consisting of four rows, three meters long, with 30.5 cm row spacing for an effective plot area of 3.66 m^2. Subplots were arranged in an augmented incomplete block design. Each incomplete block contained 20 test genotypes plus two locally adapted check genotypes (‘Jagger’ and ‘Settler CL’). Whole plots contained 15 incomplete blocks for a total of 330 plots per whole plot, 15 of which were the check genotype Jagger, and 15 of which were the check genotype Settler CL.

Weather conditions were different in the two years of the trial (Supplemental Information B). In 2012, the environment was characterized by an early, warm spring while in 2013, the environment was characterized by a late, cool, and wet spring. Rainfall from January 1 until harvest was 305 mm in 2012 and 380 mm in 2013.

2.3. Measurement of canopy spectral reflectance

CSR of each plot was measured using a dual inter-calibrated Ocean Optics USB2000 + VIS-NIR spectrometers system (Ocean Optics, Inc., Dunedin, FL) described in Stilwell et al. (2013). The spectrometers were sensitive to 2048 wavelengths in the range from 370 to 1000 nm with an optical resolution of 1.5 nm full-width half-maximum. The optical fibers were 400 micrometer low-OH VIS-NIR fibers (Ocean Optics, Inc., Dunedin, FL) with a length of 4m. Spectrometer 1 was equipped with a