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RESEARCH

Multiple Leaf Measurements Improve Effectiveness of Chlorophyll Meter for Durum Wheat Nitrogen Management

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ABSTRACT

Simple and rapid methods are needed to measure durum wheat (*Triticum durum* L.) nitrogen (N) status and make on-site N application decisions for increased crop yield and grain quality. Although chlorophyll meters (SPAD meters) have been widely tested for cereal crop N management, significant variation in SPAD meter readings among growing seasons, locations, and crop cultivars makes them challenging. Experiments with six durum wheat cultivars and six N fertilizer rates were conducted in Arizona in the 2010–2011 and 2011–2012 growing seasons to test whether multiple leaf SPAD readings on the same plants can improve estimation of crop N status by SPAD meters. The relationships between N nutrition index (NNI) and SPAD readings on the most recent fully expanded leaves (SPAD1), Sufficiency Index or normalized SPAD index (SI), Normalized difference SPAD index (NDSPAD), and the differences in SPAD readings between the second most recent and most recent fully expanded leaves (SPAD21) were compared. The results showed SPAD1 varied with growing season, growth stage, and durum wheat cultivar. All three indices, SI, NDSPAD, and SPAD21, improved the prediction of durum wheat N status compared to SPAD1. The SI measured at Feekes 10.5 or mean SI over growth stages (Feekes 5, 10, and 10.5) performed better than the other three indices in predicting crop yield. This study suggests that using SPAD21 can improve the effectiveness of the SPAD meter compared to SPAD1 and that SPAD21 can be as effective as SI without requirement of reference plots in durum wheat N management.

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Abbreviations: NDSPAD, normalized difference SPAD index; NNI, nitrogen nutrition index; SI, Sufficiency Index or normalized SPAD index; SPAD1, SPAD readings on the most recent fully expanded leaves; SPAD21, differences in SPAD readings between the second most recent and most recent fully expanded leaves.

One of the major concerns for durum wheat (*Triticum durum* L.) growers is production of high-yielding grains with high protein content to qualify for premium prices (Debaeke et al., 2006). Although N fertilizer input is high in durum wheat, growers often find it difficult to apply adequate amounts of N at the right time to produce desirable grain yield and protein content. Many growers in Arizona use fixed N fertilizer application programs to manage durum wheat crops. In these programs, N fertilizer is often under- or overapplied due to yearly variations in crop growth and N demands. During seasons of exceptionally high grain yields, N fertilizer applications from fixed programs are often not sufficient enough for the crops to attain the desirable 130 g kg^{-1} protein content (22.8 g kg^{-1} N concentration at 120 g kg^{-1} grain moisture). Conversely, when grain yield is low, N fertilizer is often overapplied in fixed programs, resulting in protein contents higher than the desirable 130 g kg^{-1} without economic gains. In both cases, the growers' profitability is reduced.

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Current recommendations for durum wheat N management in Arizona are based on preplant soil sampling and in-season tissue testing (Ottman and Thompson, 2006). However, while many growers test their soils before planting, few conduct in-season tissue analysis for N management. This is due to the fact that the vast majority of N in durum wheat is split-applied at almost every irrigation event from 3- to 4-leaf stage to flowering in Arizona. Very often, growers do not receive laboratory test results before their next N application, discouraging them from using this powerful tool. Therefore, there is a need for simple and rapid N management tools that will enable growers to estimate crop N status in the field and make on-site N application decisions (Ottman et al., 2000).

Alternative methods, such as remote sensing and image analysis, have been developed to measure crop N status instantly (Diacono et al., 2013; Thomason et al., 2011). Among these methods, instant measurements of leaf chlorophyll concentration by the chlorophyll meter (SPAD 502 meter; Minolta Camera Co., Osaka, Japan) to diagnose N deficiency are useful and require minimal equipment investment (Shapiro, 1999; Zhao et al., 2007; Denuit et al., 2002). SPAD meter readings are dimensionless measurements of the radiation absorbance by the leaf in the red and near-infrared regions (650 and 940 nm), and are closely correlated to the leaf chlorophyll concentration and N content (Debaeke et al., 2006; Reeves et al., 1993). The measurements are also linearly correlated with maximum net photosynthetic rate in wheat and soybean [*Glycine max* (L.) Merr.] (Gutiérrez-Rodríguez et al., 2000; Ma et al., 1995). For these reasons, SPAD meters have been widely used to determine crop N status, guide N management, and predict yield in many crops (Zhao et al., 2007).

In most studies on cereal crops, SPAD measurements are taken from the most recent fully expanded leaves. Ideally, SPAD readings should only change with crop N status. However, SPAD measurements on the most recent fully expanded leaf were found to vary significantly not only among cereal species, such as durum wheat, bread wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and triticale (*Triticale hexaploide* Lart.), but also among cultivars within the same species (Schepers et al., 1992; Giunta et al., 2002; Bail et al., 2005). This makes recommendation guidelines for N management less useful when different cultivars are involved. In addition, SPAD readings on the most recent fully expanded leaves also varied over crop growth stages and growing seasons (Schepers et al., 1992). Due to the variation between SPAD readings and crop N status among locations, years, and cultivars, the accuracy and usefulness of the SPAD meter as a N management tool has been questioned (Debaeke et al., 2006).

The Sufficiency Index (SI), also called normalized SPAD index, was proposed to compensate for factors other than N status that affect chlorophyll content in durum wheat, corn (*Zea mays* L.), rice (*Oryza sativa* L.), upland cotton (*Gossypium hirsutum* L.), and other crops (Blackmer and Schepers, 1995; Bronson et al., 2003; Debaeke et al., 2006; Hussain et al., 2000; Varvel et al., 1997). The SI index is the ratio of any SPAD meter reading to the maximal value measured from plants in a well-fertilized reference strip in the same field. The index was shown to be closely correlated with the durum wheat N nutrient index irrespective of year, cultivar, and growth stage, and predicted yield and protein accurately when measured at anthesis (Debaeke et al., 2006). However, this method requires establishment of high-N reference strips in the field.

The ability to predict crop yield and/or grain protein content at anthesis (Feekes 10.5) is of significant importance in durum wheat production, especially under irrigated arid conditions in Arizona. This is because the amount of N fertilizer applied at anthesis directly influences durum grain quality, including grain protein content and test weight (Ottman et al., 2000). The ability to predict crop yield and/or grain protein content will enable more informed guidance of durum wheat growers regarding the rate of the last N fertilizer application. SPAD meter readings at anthesis have been used to predict crop yield and grain protein content in both bread and durum wheat (Debaeke et al., 2006; Bail et al., 2005; Lopez-Bellido et al., 2004; Matsunaka et al., 1997). Studies have also shown that the combination of SPAD meter readings with number of spikes or leaf area index can improve the ability to predict crop yield and grain protein content (Bail et al., 2005; Nakano et al., 2010).

While many studies on SPAD meter use have been conducted in bread wheat, published data on its use in durum wheat are limited. Given the differences in leaf chlorophyll concentration and blade thickness between bread wheat and durum wheat, SPAD readings vary among bread wheat and durum wheat cultivars (Debaeke et al., 2006). In addition, almost all SPAD meter studies in durum wheat have been conducted under rain-fed Mediterranean environments (Debaeke et al., 2006; Bail et al., 2005; Lopez-Bellido et al., 2004; Yildirim et al., 2010). There are no reports on the use of SPAD meters to guide N applications, predict grain yield, and evaluate grain protein content in durum wheat in the arid conditions of the southwestern United States. Therefore, we conducted field experiments with six durum wheat cultivars and six N fertilizer rates to (i) investigate the relationship between SPAD meter readings and crop N status; (ii) examine variability in SPAD meter readings among cultivars, growth stages, and growing seasons; and (iii) test whether SPAD meter readings from multiple leaves of a single plant can be used to improve SPAD meter effectiveness in predicting durum wheat yields and/or grain protein content in an irrigated arid environment.

Table 1. Nitrogen application rate at different growth stages in durum wheat.

Growth stage	Date		N rate					
	2010–2011	2011–2012	0	73	123	185	269	403†
			kg ha^{-1}					
Preplant	not applied	8 Dec. 2011	0	0	0	0	0	90
Feekes 1–2	18 Jan. 2011	11 Jan. 2012	0	17	34	62	90	112
Feekes 5	9 Mar. 2011	28 Feb. 2012	0	11	22	34	45	56
Feekes 10	24 Mar. 2011	13 Mar. 2012	0	22	34	45	67	67
Feekes 10.5	11 Apr. 2011	9 Apr. 2012	0	22	34	45	67	78

† The rate was only included in the 2011–2012 growing season.

MATERIALS AND METHODS

Field Experiment

Field experiments were conducted at the University of Arizona's Maricopa Agricultural Center at Maricopa, AZ (33.067547°N, 111.971460°W) during the 2010–2011 and 2011–2012 growing seasons. The soil texture at the site was a Casa Grande sandy loam and sandy clay loam (fine-loamy, mixed, superactive, hyperthermic Typic Natrargid). Precipitation amounted to 29.3 and 41.1 mm in the 2010–2011 and 2011–2012 growing seasons, respectively.

The experiment with six durum wheat cultivars was arranged in a split-plot design with four replications. The main plots (cultivars) were randomly assigned within each of the four blocks. Durum wheat cultivars included Duraking, Topper, Kronos, Havasu, Orita, and Ocotillo. These cultivars are common in Arizona durum wheat production (Ottman, 2008). Preliminary analysis of samples from yield performance trials at various locations across Arizona from 1999 to 2008 showed large ranges of grain yield (7.1 to 8.1 Mg ha^{-1}) and grain N concentration (23.3 to 25.4 g N kg^{-1}) of the six cultivars used in this study (Ottman, 2008). Five N fertilizer rates were used in the 2010–2011 growing season: 0, 73, 123, 185, and 269 kg ha^{-1}. An additional N fertilizer rate of 403 kg ha^{-1} was included in the second growing season. The N fertilizer rates were assigned to subplots within durum wheat cultivars with a plot size of 5 m wide by 8 m long. Nitrogen fertilizer in the form of urea was manually applied to each plot at different growth stages according to Table 1. The fertilizer was incorporated into the soil with flood irrigation immediately after application.

Sudangrass [*Sorghum bicolor* (L.) Moench var. *sudanense* (Piper) A.S. Hitchc.] cover crops were grown in the summers before durum wheat planting to reduce excess NO_3–N from the soil and reduce variation in soil fertility. As a result, preplant soil samples showed that there was <30 kg ha^{-1} NO_3–N available in the top 90 cm of the soil profile. The last cutting of sudangrass was conducted in early September of each growing season, and fields were plowed, disked twice, and laser-leveled for durum wheat planting. To ensure N fertilizer was the only limiting factor in the study, 56 kg ha^{-1} P in the form of 0–45–0 (N–P–K) fertilizer was applied before planting. Other nutrients were sufficient according to preplant soil testing and guidelines on nutrient management of durum wheat in Arizona (Ottman and Thompson, 2006). Durum wheat was planted into dry soil on flat seed beds on 15 December in 2010 and 9 December in 2011 and irrigated immediately after planting. The planting rate was 168 kg ha^{-1} seeds with a row spacing of 19.1 cm and a planting depth of 2.5 cm.

The experimental fields were flood-irrigated to avoid water deficits according to irrigation scheduling program AZSched and periodic soil moisture measurements (Martin, 2003). The total amount of irrigation water was about 900 mm applied in nine irrigation events in both 2010–2011 and 2011–2012 growing seasons. Irrigation water provided approximately 2 kg ha^{-1} NO_3–N at each irrigation event.

Biomass and Yield Measurements

Durum wheat plants were destructively sampled from each subplot on five dates in each growing season: 18 January (Feekes 2), 24 February (Feekes 5), 22 March (Feekes 10), 7 April (Feekes 10.5), and 2 June (harvest) in 2011 and 10 January (Feekes 1), 16 February (Feekes 5), 13 March (Feekes 10), 4 April (Feekes 10.5), and 24 May (harvest) in 2012. Plants within two 0.5-m lengths of row per plot were cut at the soil surface and immediately placed in coolers. Plant biomass was then oven-dried at 65°C with ventilation to obtain the dry weight of each sample. The dried biomass was finely ground and samples were analyzed for N content with a Carlo Erba elemental analyzer (Model NA1500 N/C; Carlo Erba Instruments, Milan, Italy). The middle eight rows in each subplot were harvested with a small-plot combine harvester on 2 June 2011 and 24 May 2012. Grain yield was recorded and a sample of grain from each subplot was analyzed for grain N content. Both grain yield and grain N content were adjusted on 120 g kg^{-1} water basis.

SPAD and Crop Nitrogen Status Measurements

In the 2010–2011 growing season, SPAD meter (SPAD 502; Minolta Camera Co., Osaka, Japan) readings on different sections of leaves of different ages were collected from the Duraking cultivar on 1 Mar. 2011 (Feekes 8). Leaf selections for SPAD measurements included: (i) the most recent fully expanded leaf preceding an emerging folded leaf; (ii) the most recent fully expanded leaf preceding an emerged and halfway-unfurled new leaf; and (iii) the second most recent fully expanded leaf preceding a recently expanded leaf and an emerging folded leaf. For each leaf category, a total of 10 leaves were measured per subplot. On each selected leaf, the blade was divided into 15 sections with equal length starting from the leaf base to the tip. SPAD readings were taken from each section along the leaf blade. For example, the first section was located at 6.7% of total leaf length from the leaf base.

SPAD readings on different leaves were taken at the Feekes 5, Feekes 10, and Feekes 10.5 stages on 26 February, 23 March, and 7 April in 2011 and 14 February, 13 March, and 4 April in

2012, respectively. Readings were taken on the first and second fully expanded leaves of similar ages. SPAD readings were taken on position along leaf blade that were 60 to 70% from leaf base. A total of 15 plants were measured in each plot and the mean values of the 15 plants were used for analysis.

Data Analysis

The relationships between durum wheat biomass and plant N concentration from plots received the highest N fertilizer rate treatment (N5) in the 2010–2011 growing season and that with the two highest N fertilizer rate treatments (N5 and N6) in the 2011–2012 growing season were analyzed according to the dilution law (Greenwood et al., 1990). The following equation was fitted to shoot N concentration (g kg^{-1}) and plant aboveground dry mass (DM, Mg ha^{-1}) data:

$$\mathrm{Nc} = a \times \mathrm{DM}^{-b} \quad [1]$$

where Nc is the critical shoot N concentration at which additional N uptake no longer increases plant growth, and a and b are parameters.

Using the relationship between Nc and plant biomass, the N nutrition index (NNI) was calculated for each plot by dividing actual N concentration data by Nc. A value of NNI = 1 indicates plants have optimal N status and a value of NNI < 1 indicates N deficiency in plants (Debaeke et al., 2006).

Quadratic equations were fitted to SPAD readings from the different position along the leaf blade as dependent variable and leaf blade position (leaf base as 0% and leaf tip as 100%) as independent variable. From each equation, the highest SPAD reading on leaves and the position along leaf blade with the reading were calculated for each type of leaf age.

The SI was calculated as the ratio of any SPAD reading over the SPAD reading from the treatment with highest N rate for each year according to Debaeke et al. (2006). The normalized difference SPAD (NDSPAD) was calculated according to Zhao et al. (2007). In addition, differences between the SPAD readings of the second and first fully expanded leaves (SPAD21) were also calculated. These SPAD indices are calculated as follows:

$$\mathrm{SI} = \frac{\mathrm{SPAD1}}{\mathrm{SPAD1\ in\ the\ treatment\ with\ highest\ N\ rate}} \quad [2]$$

$$\mathrm{NDSPAD} = \frac{\mathrm{SPAD2} - \mathrm{SPAD1}}{\mathrm{SPAD1} + \mathrm{SPAD2}} \quad [3]$$

$$\mathrm{NDSPAD} = \mathrm{SPAD2} - \mathrm{SPAD1} \quad [4]$$

where SPAD1 and SPAD2 are SPAD readings of the first and second most recent fully expanded leaf, respectively.

Linear regression lines were fitted to the relationships between the above SPAD indices and the logarithmically transformed values of NNI. The GLM procedure with the "solution" option in SAS (SAS Inst. Inc., Cary, NC) was used to identify significant variations among growing seasons, growth stages, or cultivars. The slope and/or intercepts of different lines were also compared using the method. The procedure is based on the principle of extra sum of squares described in Ratkowsky (1983).

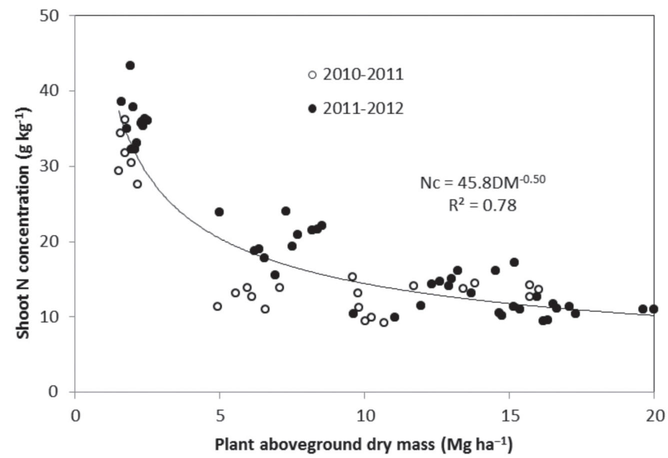

Figure 1. Relationship of plant aboveground dry mass (DM, Mg ha^{-1}) and shoot N concentration (Nc, g kg^{-1}) in durum wheat in plots with the highest N fertilizer rate treatment (N5) in the 2010–2011 growing season and that with the two highest N fertilizer rate treatments (N5 and N6) in the 2011–2012 growing season.

Exponential equations were used to describe the relationships between SPAD indices at Feekes 10.5 and crop yield. When the means of the SPAD indices at Feekes 5, Feekes 10, and Feekes 10.5 were used, linear regression was used to analyze the relationships between SPAD indices and crop yield. In all cases, scatterplots were used to determine the trend in the data and the equations with minimal parameters and higher R^2 were chosen to fit the data.

RESULTS

Relationships between Aboveground Biomass and Critical Nitrogen Concentration

As durum wheat crop biomass increased, N concentration decreased (Fig. 1). The critical shoot N concentration (Nc, g kg^{-1}) and shoot dry mass (DM, Mg ha^{-1}) in the high-N treatments (the N5 treatment in 2010–2011 and the N5 and N6 treatments in 2011–2012) showed a power relationship of Nc = 45.8 $DM^{-0.50}$ with an R^2 of 0.78. The parameters in the equation obtained from our results are slightly different from the previously published values, 53.5 and 0.44, probably due to different environments and crop management (Justes et al., 1994, 1997).

SPAD Readings Affected by Leaf Age and Positions along Leaf Blade

In the 2010–2011 growing season, SPAD meter readings were taken from leaves with different ages and from different positions along the leaf blade for the Duraking cultivar. Because durum wheat leaves emerge and unfurl over a substantial period of time, the leaf blade age varies considerably from the base to the tip. Thus, SPAD readings taken from different positions along the leaf blade varied significantly (Fig. 2). SPAD readings along the leaf blade were lower at positions closest to the ligule or leaf base, increased with distance away from the leaf base, and reach the maximum at 60 to 70% from leaf base (Table

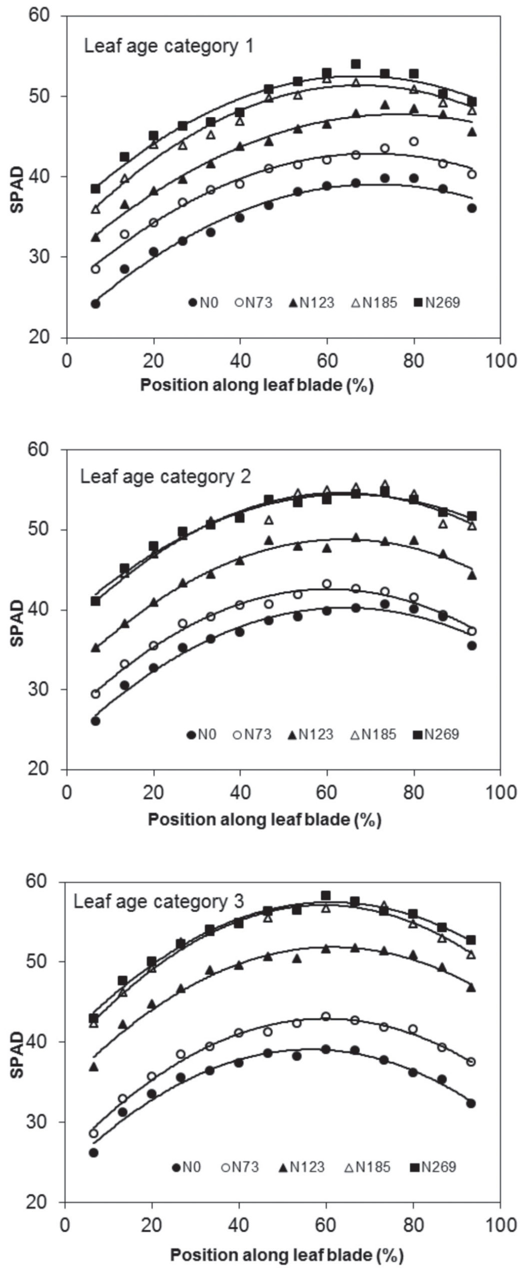

Figure 2. Relationship of SPAD readings and leaf age or position along leaf blade in durum wheat. Leaf age Category 1 includes the most recent fully expanded leaf preceding an emerging folded leaf; leaf age Category 2 includes the most recent fully expanded leaf preceding an emerged and halfway-unfurled new leaf; leaf age Category 3 includes the second most recent fully expanded leaf preceding a recently expanded leaf and an emerging folded leaf. On each selected leaf, blade was divided into 15 sections with equal length starting from the leaf base to the tip. Leaf base was treated as 0% and leaf tip as 100%. N0, N73, N123, N185, N269 = nitrogen application rates of 0, 73, 123, 185, and 269 kg ha^{-1}.

2). Readings on leaves from Category 1 (the most recent fully expanded leaf preceding an emerging folded leaf) were highest when taken at 67 to 76% of the total leaf length from leaf base, 60 to 65% for leaves in Category 2 (the most recent fully expanded leaf preceding an emerged and halfway-unfurled new leaf), and 56 to 61% for leaves in Category 3 (the second most recent fully expanded leaf preceding a recently expanded leaf and an emerging folded leaf). This indicates that both leaf age and position along leaf blade affect SPAD meter readings in the field.

The SPAD readings generally increased with leaf age (Table 2). The SPAD reading differences between the leaves in Category 2 and Category 1 were 1.2, −0.3, 1.1, 3.4, and 2.0 SPAD units for the N rate of 0, 73, 123, 185, and 269 kg ha^{-1}, respectively. This indicates that leaf age is an important factor that needs to be considered when collecting SPAD measurements. The differences between the Category 3 and Category 1 readings were 0, 0.1, 4.2, 5.8, and 5.0 SPAD units for the N rate of 0, 73, 123, 185, and 269 kg ha^{-1}, respectively, indicating that plants from the higher N rate can keep leaves in the lower position greener relative to the first fully expanded leaves. This also indicates that it is possible to correlate the differences between the leaves from different positions with crop N status.

Relationships between Various SPAD Indices and Nitrogen Nutrition Index

The SPAD readings had a linear relationship with log-transformed values of NNI (Fig. 3). The regression line of SPAD meter readings from the first fully expanded leaf and ln(NNI) had an R^2 of 0.36. The SI explained significantly higher variation in ln(NNI) with an R^2 of 0.57, indicating that the use of a high-N reference can significantly improve the effectiveness of SPAD readings to guide N applications. When SPAD readings from the second leaf were used as reference, the regression equations of NDSPAD and SPAD21 with ln(NNI) explained 53 and 57% of variation in ln(NNI), respectively. This indicates that using the second leaf as a reference could also improve the effectiveness of SPAD meter readings without requiring high-N reference strips in the field. Since an NNI value of 1 [when ln(NNI) = 0] indicates the readings at which there is sufficient N in durum wheat plants, our studies suggest that durum wheat crops reach sufficient N status when values of SPAD1, SI, NDSPAD, and SPAD21 are >48.90, 0.98, 0.028, and 2.75, respectively, in the growing season.

Variations in SPAD Reading over Growing Seasons

The relationships between SPAD1, SI, NDSPAD, and SPAD21 with ln(NNI) over growing seasons are shown in Table 3. The critical points at which durum wheat plants have sufficient N were obtained when ln(NNI) was zero. When the variations in this critical point were low over growing seasons, it is possible that a universal value can be used across growing seasons.

There were significant differences in the SPAD1–NNI relationship between the two growing seasons. However, when the data from both growing seasons were combined,

Table 2. SPAD readings affected by leaf age and position along leaf blade in durum wheat.

N rate	Leaf age Category 1† Position along leaf blade with highest SPAD reading‡	Leaf age Category 1† Highest SPAD reading on leaf blade	Leaf age Category 2 Position along leaf blade with highest SPAD reading	Leaf age Category 2 Highest SPAD reading on leaf blade	Leaf age Category 3 Position along leaf blade with highest SPAD reading	Leaf age Category 3 Highest SPAD reading on leaf blade
kg ha^{-1}	%		%		%	
0	71.2	39.1	64.2	40.3	56.3	39.1
73	70.2	42.9	60.4	42.6	59.8	43.0
123	75.9	47.7	63.7	48.8	61.3	51.9
185	67.5	51.3	63.1	54.7	59.3	57.1
269	67.1	52.5	64.7	54.5	60.9	57.5
Mean	70.4		63.2		59.5	

† Leaf age Category 1 includes the most recent fully expanded leaf preceding an emerging folded leaf; leaf age Category 2 includes the most recent fully expanded leaf preceding an emerged and halfway-unfurled new leaf; leaf age Category 3 includes the second most recent fully expanded leaf preceding a recently expanded leaf and an emerging folded leaf. Leaf age from older to younger was Category 3 > Category 2 > Category 1.

‡ On each selected leaf, blade was divided into 15 sections with equal length starting from the leaf base to the tip. Leaf base was treated as 0% and leaf tip as 100%.

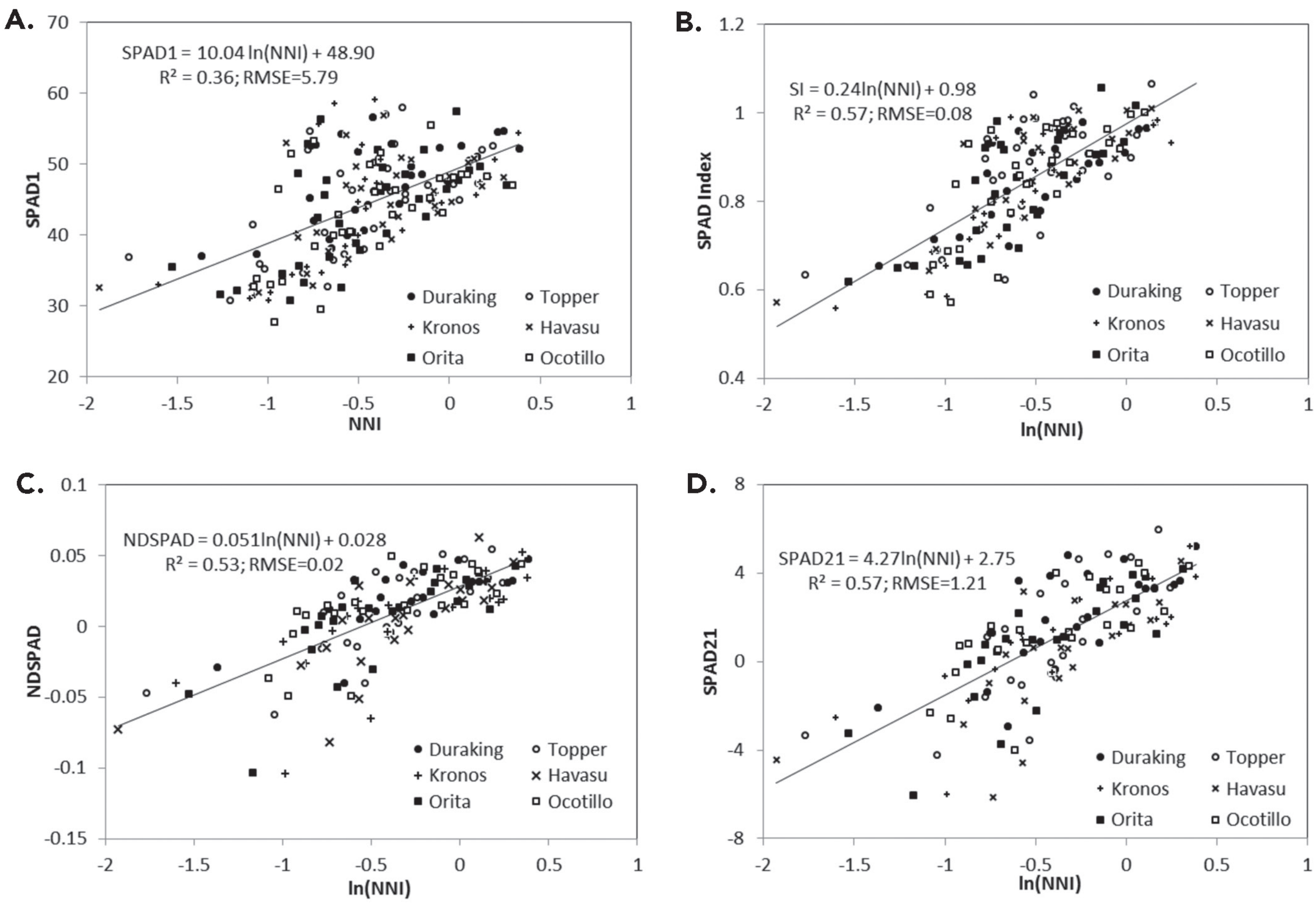

Figure 3. Relationship of SPAD indices and nitrogen nutrition index (NNI) in durum wheat cultivars: (a) SPAD readings on the most recent fully expanded leaves (SPADL1); (b) Sufficiency Index or normalized SPAD index (SI); (c) normalized difference SPAD index (NDSPAD); (d) differences in SPAD readings between the second most recent and most recent fully expanded leaves (SPAD21).

the SPAD1–NNI relationships differed from those observed when either season was analyzed separately (Table 3). Although high-N references were used, the SI did not reduce variations between the growing seasons. The SI–NNI relationships for each of the growing seasons were different from those obtained when the data from both growing seasons were combined. At the same time, NDSPAD reduced the variation in NDSPAD–NNI relationship among growing seasons significantly. Only the intercept (the NDSPAD value to reach critical shoot N concentration) in the 2010–2011 growing season was different from that when the data from both growing seasons were combined. SPAD21 reduced variations among the years, indicating the stability of this index over growing seasons.

Table 3. Stability of the functions of SPAD and nitrogen nutrition index (NNI) at 2010–2011 and 2011–2012 growing seasons combined across growth stages and durum wheat cultivars.

Index†	Growth stage	Slope	Critical value‡	95% interval of critical value	R^2
SPAD1§	2010–2011	13.47§	54.03 a§¶	±2.59	0.42
	2011–2012	13.44§	47.49 b	±0.88	0.65
	Combined	10.04	48.90	±1.18	0.36
SI	2010–2011	0.31 a§	1.07 a§	±0.041	0.71
	2011–2012	0.29 b	0.96 b§	±0.020	0.69
	Combined	0.24	0.98	±0.021	0.57
NDSPAD	2010–2011	0.051	0.037 a§	±0.010	0.76
	2011–2012	0.059	0.028 b	±0.005	0.50
	Combined	0.051	0.028	±0.005	0.53
SPAD21	2010–2011	4.10	3.37	±1.08	0.65
	2011–2012	5.10	2.77	±0.40	0.57
	Combined	4.27	2.75	±0.38	0.57

† SPAD1, SPAD readings on the most recent fully expanded leaves; SI, Sufficiency Index or normalized SPAD index (SPAD1/SPAD1 in the treatment with highest N rate); NDSPAD, normalized difference SPAD index ([SPAD2 − SPAD1]/[SPAD2 + SPAD1]); SPAD21, differences in SPAD readings between the second most recent and most recent fully expanded leaves.

‡ Critical values are the value of a particular index at which additional N uptake no longer increases plant growth (NNI = 1).

§ Indicates significant difference in the regression parameters between models from individual year and those of combined data.

¶ Within each column and each index, means between years followed by the same letter are not significantly different according to extra sum of squares test ($p = 0.05$).

Table 4. Stability of the functions of SPAD and nitrogen nutrition index (NNI) at Feekes 5, Feekes 10, and Feekes 10.5 stages combined across growing seasons and durum wheat cultivars.

Index†	Growth stage	Slope	Critical value‡	95% interval of critical value	R^2
SPAD1	Feekes 5	13.96 a§¶	47.51 b	±0.81	0.85
	Feekes 10	10.70 ab	47.82 b	±1.72	0.42
	Feekes 10.5	9.81 b	53.37 a§	±2.36	0.37
	Combined	10.04	48.90	±1.18	0.36
SI	Feekes 5	0.27	0.97 b	±0.022	0.81
	Feekes 10	0.26	0.96 b§	±0.034	0.60
	Feekes 10.5	0.24	1.03 a§	±0.044	0.59
	Combined	0.24	0.98	±0.021	0.57
NDSPAD	Feekes 5	0.027 b§	0.020 b	±0.004	0.52
	Feekes 10	0.037 ab	0.035 a§	±0.005	0.52
	Feekes 10.5	0.056 a	0.026 b	±0.010	0.51
	Combined	0.051	0.028	±0.005	0.53
SPAD21	Feekes 5	2.62 b	1.97 b	±0.37	0.59
	Feekes 10	3.97 ab	3.38 a§	±0.41	0.65
	Feekes 10.5	4.48 a	2.60 b	±0.82	0.51
	Combined	4.27	2.75	±0.38	0.57

† SPAD1, SPAD readings on the most recent fully expanded leaves; SI, Sufficiency Index or normalized SPAD index (SPAD1/SPAD1 in the treatment with highest N rate); NDSPAD, normalized difference SPAD index ([SPAD2 − SPAD1]/[SPAD2 + SPAD1]); SPAD21, differences in SPAD readings between the second most recent and most recent fully expanded leaves.

‡ Critical value are the value of a particular index at which additional N uptake no longer increases plant growth (NNI = 1).

§ Within each column and each index, means among stages followed by the same letter are not significantly different according to extra sum of squares test ($p = 0.05$).

¶ Indicates significant difference in the regression parameters between models from individual stage and those of combined data.

Variations in SPAD Meter Measurements over Growth Stages

The relationships between SPAD1, SI, NDSPAD, and SPAD21 with NNI changed over growth stages (Table 4). For all four indices, there was at least one growth stage among Feekes 5, Feekes 10, and Feekes 10.5 stage at which the relationship of these indices with NNI was different compared to that when the data from the three growth stages were combined. For SPAD1 and SI, the intercepts of the linear equations of these indices with NNI at Feekes 10.5 were higher compared to those at the Feekes 5 and Feekes 10 stages. For NDSPAD and SPAD21, the intercepts at Feekes 10 were higher than at the Feekes 5 and Feekes 10.5 stages. The SPAD1 measurements changed over growth stages, especially in the 2010–2011 growing season (data not shown). This probably resulted in a lower R^2 for the relationship between SPAD1 with NNI when the data from three growth stages were combined (Table 4).

Variations in SPAD Measurements among Cultivars

The differences in SPAD measurements among durum wheat cultivars were less significant compared to measurements over growing seasons and growth stages (Table 5). The Duraking cultivar had a different relationship between SPAD1 and ln(NNI) compared to the combined data with all six cultivars, whereas the Havasu cultivar had a different relationship between SPAD21 and ln(NNI) compared to the combined data. There were no differences among cultivars in the slope of the relationship of these indices with ln(NNI). However, significant differences in the intercept of the relationships were observed among the cultivars for SPAD1, NDSPAD, and SPAD21, indicating that different values should be used for the critical plant N status of each cultivar. However, the SI minimized the differences in crop cultivars due to the use of a high-N reference. The SI, NDSPAD, and SPAD21 had significantly higher R^2 compared to SPAD1, indicating that these indices can increase effectiveness of SPAD readings compared to SPAD1 and that SPAD1 was less stable across different crop cultivars when used for predicting plant N status.

Using SPAD Indices to Predict Durum Wheat Yield and Grain Nitrogen Content

The relationships between the four indices at Feekes 10.5 and durum wheat yield were described by exponential curves (Table 6). The SI had the highest R^2 value and SPAD1 had the lowest R^2 among the indices, indicating that the use of well-fertilized N plots and yield potential data could significantly increase the ability to predict durum wheat yield. The R^2 values for the relationships between durum wheat yield

Table 5. Stability of the functions of SPAD and nitrogen nutrition index (NNI) with different durum wheat cultivars combined across growing seasons and growth stages.

Index†	Cultivar	Slope	Critical value‡	95% interval of critical value	R^2
SPAD1	Duraking	9.35	50.74 a§	±2.18	0.40
	Topper	9.55	49.50 ab	±3.10	0.33
	Kronos	10.49	48.18 b	±3.29	0.37
	Havasu	8.89	47.81 b	±2.72	0.34
	Orita	10.54	48.81 ab	±3.25	0.36
	Ocotillo	10.62	47.97 b	±3.04	0.36
	Combined	10.04	48.90	±1.18	0.36
SI	Duraking	0.19	0.96	±0.045	0.48
	Topper	0.20	0.96	±0.057	0.50
	Kronos	0.25	0.96	±0.049	0.69
	Havasu	0.23	0.98	±0.043	0.68
	Orita	0.26	0.99	±0.063	0.58
	Ocotillo	0.28	0.99	±0.059	0.58
	Combined	0.24	0.98	±0.021	0.57
NDSPAD	Duraking	0.039	0.030 ab	±0.008	0.50
	Topper	0.055	0.032 a	±0.012	0.59
	Kronos	0.049	0.022 ab	±0.015	0.48
	Havasu	0.056	0.021 b	±0.012	0.62
	Orita	0.056	0.030 ab	±0.013	0.57
	Ocotillo	0.046	0.032 a	±0.012	0.49
	Combined	0.051	0.028	±0.005	0.53
SPAD21	Duraking	3.81	3.20 a	±0.82	0.51
	Topper	4.84	3.08 a	±1.06	0.57
	Kronos	3.81	2.37 ab	±0.82	0.65
	Havasu	4.43	1.99 b	±1.04	0.56
	Orita	4.61	2.79 ab	±0.92	0.64
	Ocotillo	3.95	2.99 a	±0.90	0.55
	Combined	4.27	2.75	±0.38	0.57

† SPAD1, SPAD readings on the most recent fully expanded leaves; SI, Sufficiency Index or normalized SPAD index (SPAD1/SPAD1 in the treatment with highest N rate); NDSPAD, normalized difference SPAD index ([SPAD2 − SPAD1]/[SPAD2 + SPAD1]); SPAD21, differences in SPAD readings between the second most recent and most recent fully expanded leaves.

‡ Critical value are the value of a particular index at which additional N uptake no longer increases plant growth (NNI = 1).

§ Within each column and each index, means among varieties followed by the same letter are not significantly different according to extra sum of squares test (p = 0.05).

and NDSPAD or SPAD21 were slightly higher compared to that for the relationship between crop yield and SPAD1.

The mean of these indices over growth stages had a linear relationship with durum yield (Table 6). Using average SPAD readings over different growth stages to predict crop yield has an advantage over using SPAD measurements at a single stage because the crop N status at one stage might not be representative over the whole growing season. The mean of the SI over growth stages had the highest R^2 value with durum wheat yield of all the four indices. The means of the NDSPAD and SPAD21 indices with crop yield, however, produced slightly lower R^2 values relative to that of the SPAD1.

DISCUSSION

Arizona durum wheat producers rarely use recommended in-season tissue analysis to guide their N applications because of the practical issues. This calls for instant N diagnosis indicators that are specific, sensitive, practical, and able to predict crop N status during the growing season (Meynard et al., 1997). While SPAD meter readings on the most recent fully expanded leaf have been widely used in cereal crops to predict plant N status, crop yield, and grain quality, studies have shown that results vary with year, location, and cultivar (Debaeke et al., 2006; Schepers et al., 1992; Giunta et al., 2002; Bail et al., 2005; Shapiro, 1999; Zhao et al., 2007). The results from this study are in agreement with previous publications and this impacts the usefulness of the SPAD meter as an N fertilizer management tool. In this study, three indices derived from SPAD meter readings are proposed to improve the effectiveness of SPAD meters for durum wheat N management.

The SI or normalized SPAD index, a ratio of SPAD value of a particular plot to that of a well-fertilized reference plot in the same field, was proposed to reduce these variations in durum and bread wheat, rice, upland cotton, corn, and other crops (Blackmer and Schepers, 1995; Bronson et al., 2003; Debaeke et al., 2006; Hussain et al., 2000; Piekielek et al., 1995; Varvel et al., 1997). In durum wheat, this index correlated closely with plant N status irrespective of year, cultivar, and growth stage (Debaeke et al., 2006). In our study, the SI minimized variations in predicting crop N status among cultivars and reduced the variations among growing seasons and growth stages when SPAD readings on the most recent fully expanded leaves were compared. The SI at Feekes 10.5 and mean SI over three growth stages (Feekes 5, Feekes 10, and Feekes 10.5) had the highest R^2 value in predicting durum wheat yield of all SPAD indices tested in this study. This indicates that a combination of SPAD readings and high-N reference strips is a powerful tool to improve the effectiveness of the SPAD meter use in N fertilizer management in durum wheat.

The use of the SI fits particularly well into regions where high-N reference strips are deployed (Shanahan et al., 2008). However, in areas where high-N reference strips are not used, growers may be required to set them up for every cultivar, field, and planting date. This extra requirement may significantly reduce adoption of this method by growers.

The use of differences between SPAD readings from the first and second most recent fully expanded leaves could also improve effectiveness of SPAD readings without the need for N reference strips. Zhao et al. (2007) proposed the NDSPAD to guide variable-rate N application in bread wheat. The NDSPAD explained 65% of the variations in plant N uptake. In our study, the NDSPAD reduced variation in predicting durum wheat N status compared to SPAD readings on the first fully expanded

Table 6. Relationship of SPAD indices at Feekes 10.5 and mean SPAD indices over the growing seasons with durum wheat grain yield.

Index†	SPAD indices at Feekes 10.5	R^2	Mean value of SPAD indices over growing stages	R^2
SPAD1	Yield = $0.18e^{0.07\times SPAD1}$	0.69	Yield = 328.2SPAD1 − 9394	0.77
SI	Yield = $0.12e^{4.02\times SI}$	0.83	Yield = 17,384SI − 10,094	0.85
NDSPAD	Yield = $5.27e^{13.0\times NDSPAD}$	0.74	Yield = 74,511NDSPAD + 4850	0.71
SPAD21	Yield = $4.65e^{0.16\times SPAD21}$	0.71	Yield = 855.6SPAD21 + 4543	0.70

† SPAD1, SPAD readings on the most recent fully expanded leaves; SI, Sufficiency Index or normalized SPAD index (SPAD1/SPAD1 in the treatment with highest N rate); NDSPAD, normalized difference SPAD index ([SPAD2 − SPAD1]/[SPAD2 + SPAD1]); SPAD21, differences in SPAD readings between the second most recent and most recent fully expanded leaves.

leaves. This index could also be a useful tool for in-season N management in durum wheat, and it does not require extra N-rich plots in the field.

While differences in SPAD readings between the fourth and the first fully expanded leaves have been proposed to manage N input in cotton (Qu et al., 2007), the use of differences in SPAD readings between leaves has yet to be proposed in durum wheat N management. In this study, SPAD21 had a comparable R^2 value with the SI and NDSPAD in predicting plant N status, indicating the index can also be used for in-season N management. Additionally, the SPAD21 is simple and easy to compute in the field and could become a practically useful index to improve the effectiveness of SPAD meter use for estimating durum wheat N status and making on-site N application decisions. Using leaf at lower position as references also makes it possible to produce one single indicator for durum wheat N management regardless of durum wheat variety, growth stage, and growing seasons. This could increase the usefulness and effectiveness of SPAD meters in crop N management.

Our study also showed that the leaf age and the position along the leaf blade should also be considered to reduce variations since SPAD readings were dependent on leaf age and the position along the leaf blade. The similar pattern was observed in corn and the results are consistent with spatial distributions and net deposition rates of mineral elements in the elongating bread wheat leaf (Vig et al., 2012; Hu and Schmidhalter, 1998). Given the large differences in SPAD meter readings at different positions, measurements should be taken from the same position along the leaf blade with similar leaf age for any indices in this study to be useful. Measurements from as many plants as possible are also recommended to reduce the effects of plant to plant variations on the indices.

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References

Bail, M.L., M.H. Jeuffroy, C. Bouchard, and A. Barbottin. 2005. Is it possible to forecast the grain quality and yield of different varieties of winter wheat from Minolta SPAD meter measurements? Eur. J. Agron. 23:379–391. doi:10.1016/j.eja.2005.02.003

Blackmer, T.M., and J.S. Schepers. 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J. Prod. Agric. 8:56–60. doi:10.2134/jpa1995.0056

Bronson, K.F., T.T. Chua, J.D. Booker, J.W. Keeling, and R.J. Lascano. 2003. In-season nitrogen status sensing in irrigated cotton: II. Leaf nitrogen and biomass. Soil Sci. Soc. Am. J. 67:1439–1448. doi:10.2136/sssaj2003.1439

Debaeke, P., P. Rouet, and E. Justes. 2006. Relationship between the normalized SPAD index and the nitrogen nutrition index: Application to durum wheat. J. Plant Nutr. 29:75–92. doi:10.1080/01904160500416471

Denuit, J.P., M. Olivier, M.J. Goffaux, J.L. Herman, J.P. Goffart, J.P. Destain, and M. Frankinet. 2002. Management of nitrogen fertilization of winter wheat and potato crops using the chlorophyll meter for crop nitrogen status assessment. Agronomie 22:847–853. doi:10.1051/agro:2002065

Diacono, M., P. Rubino, and F. Montemurro. 2013. Precision nitrogen management of wheat. A review. Agron. Sustainable Dev. 33:219–241. doi:10.1007/s13593-012-0111-z

Giunta, F., R. Motzo, and M. Deidda. 2002. SPAD readings and associated leaf traits in durum wheat, barley and triticale cultivars. Euphytica 125:197–205. doi:10.1023/A:1015878719389

Greenwood, D.J., G. Lemaire, G. Gossef, P. Cruz, A. Draycott, and J.J. Neeteson. 1990. Decline in percentage N of C_3 and C_4 crops with increasing plant mass. Ann. Bot. (Lond.) 66:425–436.

Gutiérrez-Rodríguez, M., M.P. Reynolds, and A. Larqué-Saavedra. 2000. Photosynthesis of wheat in a warm, irrigated environment II. Traits associated with genetic gains in yield. Field Crops Res. 66:51–62. doi:10.1016/S0378-4290(99)00078-7

Hu, Y., and U. Schmidhalter. 1998. Spatial distributions and net deposition rates of mineral elements in the elongating wheat (*Triticum aestivum* L.) leaf under saline soil conditions. Planta 204:212–219. doi:10.1007/s004250050249

Hussain, F., K.F. Bronson, Y. Singh, B. Singh, and S. Peng. 2000. Use of chlorophyll meter efficiency indices for nitrogen management of irrigated rice in Asia. Agron. J. 92:875–879.

Justes, E., M.H. Jeuffroy, and B. Mary. 1997. Wheat, barley, and durum wheat. In: G. Lemaire, editor, Diagnosis of the nitrogen status in crops. Springer Verlag, Berlin. p. 73–91.

Justes, E., B. Mary, J.M. Meynard, J.M. Machet, and L. Thelier-Huche. 1994. Determination of critical nitrogen dilution curve for winter wheat crops. Ann. Bot. (Lond.) 74:397–407. doi:10.1006/anbo.1994.1133

Lopez-Bellido, R.J., C.E. Shepherd, and P.B. Barraclough. 2004. Predicting postanthesis N requirements of bread wheat with a Minolta SPAD meter. Eur. J. Agron. 20:313–320. doi:10.1016/S1161-0301(03)00025-X

Ma, B.L., M.J. Morrison, and H.D. Voldeng. 1995. Leaf greenness and photosynthetic rates in soybean. Crop Sci. 35:1411–1414. doi:10.2135/cropsci1995.0011183X003500050025x

Martin, E.C. 2003. Arizona irrigation scheduling system (AZSched). Univ. of Arizona Coop. Ext. http://ag.arizona.edu/crop/irrigation/azsched/azsched.html (accessed 27 Nov. 2012).

Matsunaka, T., Y. Watanabe, T. Miyawaki, and N. Ichikawa. 1997. Prediction of grain protein content in winter wheat through leaf color measurements using a chlorophyll meter. Soil Sci. Plant Nutr. 43:127–134. doi:10.1080/00380768.1997.10414721

Meynard, J.M., C. Aubry, E. Justes, and M. Le Bail. 1997. Nitrogen diagnosis and decision support. In: G. Lemaire, editor, Diagnosis of the nitrogen status in crops. Springer Verlag, Berlin. p. 147–161.

Nakano, H., S. Morita, O. Kusuda, and Y. Sasaki. 2010. Leaf blade dry weight and leaf area index×SPAD value at anthesis can be used to estimate nitrogen application rate at anthesis required to obtain target protein content of grain in bread wheat. Plant Prod. Sci. 13:297–306. doi:10.1626/pps.13.297

Ottman, M.J. 2008. Survey of durum production practices, 2007. The 2008 Forage and Grain Report. Univ. of Arizona. http://cals.arizona.edu/pubs/crops/az1459/az1459a1.pdf (accessed 27 Nov. 2012).

Ottman, M.J., T.A. Doerge, and E. Martin. 2000. Durum grain quality as affected by nitrogen fertilization near anthesis and irrigation during grain fill. Agron. J. 92:1035–1041. doi:10.2134/agronj2000.9251035x

Ottman, M., and T. Thompson. 2006. Fertilizing small grains in Arizona. Univ. of Arizona Coop. Ext. Bull. AZ1346. http://cals.arizona.edu/pubs/crops/az1346.pdf (accessed 27 Nov. 2012).

Piekielek, W.P., R.H. Fox, J.D. Toth, and K.E. Macneal. 1995. Use of a chlorophyll meter at the early dent stage of corn to evaluate nitrogen sufficiency. Agron. J. 87:403–408. doi:10.2134/agronj1995.00021962008700030003x

Qu, W.Q., S.H. Wang, B.L. Chen, Y.H. Wang, and Z.G. Zhou. 2007. SPAD value of cotton leaves on main stem and nitrogen diagnosis for cotton growth. Acta Agron. Sin. 33:1010–1017.

Ratkowsky, D.A. 1983. Comparing parameter estimates from more than one data set. In: D.A. Ratkowsky, editor, Nonlinear regression modeling. Marcel Dekker, New York. p. 135–154.

Reeves, D.W., P.L. Mask, C.W. Wood, and D.P. Delaney. 1993. Determination of wheat nitrogen status with hand-held chlorophyll meter: Influence of management practices. J. Plant Nutr. 16:781–796. doi:10.1080/01904169309364574

Schepers, J.S., D.D. Francis, M. Vigil, and F.E. Below. 1992. Comparison of corn leaf nitrogen concentration and chlorophyll meter readings. Commun. Soil Sci. Plant Anal. 23:2173–2187. doi:10.1080/00103629209368733

Shanahan, J.F., N.R. Kitchen, W.R. Raun, and J.S. Schepers. 2008. Responsive in-season nitrogen management for cereals. Comput. Electron. Agric. 61:51–62. doi:10.1016/j.compag.2007.06.006

Shapiro, C.A. 1999. Using a chlorophyll meter to manage nitrogen applications to corn with high nitrate irrigation water. Commun. Soil Sci. Plant Anal. 30:1037–1049. doi:10.1080/00103629909370266

Thomason, W.E., S.B. Phillips, P.H. Davis, J.G. Warren, M.M. Alley, and M.S. Reiter. 2011. Variable nitrogen rate determination from plant spectral reflectance in soft red winter wheat. Precis. Agric. 12:666–681. doi:10.1007/s11119-010-9210-5

Varvel, G.E., J.S. Schepers, and D.D. Francis. 1997. Ability for in-season correction of nitrogen in corn using chlorophyll meters. Soil Sci. Soc. Am. J. 61:1233–1239. doi:10.2136/sssaj1997.03615995006100040032x

Vig, R., L. Huzsvai, A. Dobos, and J. Nagy. 2012. Systematic measurement methods for the determination of the SPAD values of maize (*Zea mays* L.) canopy and potato (*Solanum tuberosum* L.). Commun. Soil Sci. Plant Anal. 43:1684–1693. doi:10.1080/00103624.2012.681740

Yildirim, M., H. Kiliç, E. Kendal, and T. Karahan. 2010. Applicability of chlorophyll meter readings as yield predictor in durum wheat. J. Plant Nutr. 34:151–164. doi:10.1080/01904167.2011.533319

Zhao, C., A. Jiang, W. Huang, K. Liu, L. Liu, and J. Wang. 2007. Evaluation of variable–rate nitrogen recommendation of winter wheat based on SPAD chlorophyll meter measurement. N. Z. J. Agric. Res. 50:735–741. doi:10.1080/00288230709510345