

Evaluation of SPAD chlorophyll fluorescence for on-site nitrogen assessment in drip fertigated sweet corn

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Abstract

This study was conducted over two growing seasons to evaluate the potential use of the Minolta SPAD-502 chlorophyll meter for rapid onsite determination of plant N status in a drip-fertigated nitrogen cropping regime of sweet corn. Four N fertilizer rates (0, 84, 168, and 336 kg N ha⁻¹) were applied to two sweet corn cultivars ('Kandy Plus' and 'Sugar Ace') in a factorial split-plot design. Leaf SPAD and leaf total N concentration were measured at 4 different growth stages (seventh leaf stage (V7), tenth leaf stage V10), silking (R1) and milking (R3) in season one and three sampling stages (V6, V10 and R1) in 2002. In season two, leaf N concentrations and leaf 'SPAD chlorophyll' measurements increased early in the season up to silking (R1 growth stage) and declined thereafter. Leaf N concentrations and leaf SPAD readings were positively correlated. Critical SPAD values (readings associated with the lowest N fertilizer rate and providing the highest marketable ear yield) were estimated as 52.8, 52.1 and 59.0 at V7, V10 and R1 in season one; in season two the values were 51.0, 49, and 56.5 respectively. Regressing N rate on yield placed maximum ear yields at 13.8 tons ha⁻¹ with 174.8 kg N ha⁻¹ in season one and 9.7 tons ha⁻¹ with 306.2 kg ha⁻¹ in season two. Increase in leaf SPAD readings at silking reflects a strong sink/source relationship among leaves for absorbed N. The positive relationship between leaf SPAD readings and leaf N status is due to the responsiveness of SPAD-chlorophyll to N fertilizer application. These results indicate that the SPAD meter can be used effectively to assess sweet corn N status early in the season, and at this time it is possible to correct any N deficiencies that might compromise yields.

Key words: Sweet corn, SPAD-chlorophyll, N-fertilizer, silking

Introduction

Nitrogen (N) is an essential nutrient required for normal growth and development of plants as it is an integral component of the chlorophyll molecule, the principal site of light absorption necessary for photosynthesis (Taiz and Zeiger, 1991). Specific N recommendations for sweet corn vary with soil type, climatic conditions, and cultural practices (Swiader and Ware, 2002). The recommended N rates for sweet corn range from 84 kg N ha⁻¹ on silty clay loams to more than 224 kg N ha⁻¹ on highly leached sandy soils (Swiader and Ware, 2002). For more efficient use of N in irrigated cropping systems, the rate is usually split into several small applications during the growing season. In the Midwest of USA, a preplant N rate of 67 kg ha⁻¹ is recommended, but can be adjusted depending on the soil type, previous management, and soil organic matter content (Foster *et al.*, 2002).

Fresh market sweet corn growers aim at maintaining high soil N levels late into the season, since consumers expect sweet corn with dark green husks and long ears. N fertilization of high-value vegetable crops such as sweet corn has historically been managed with the goal of maximum productivity rather than maximum efficiency. However, the practice has negative environmental consequences, particularly regarding nitrate (NO₃⁻) pollution of surface and ground water. This has provided impetus for more careful and introspective N management in vegetable production, thus making N fertilizer-use efficiency a major goal of the Illinois vegetable industry. Unfortunately, accurate prediction of soil N contribution is difficult because it varies with

soil type and environmental conditions such as moisture regimes and temperature, which are beyond the growers' control. In the context of these concerns and issues, it is important that vegetable producers have at their disposal effective and environmentally sound N management systems that produce high quality food with minimum inputs. Growers need a reliable on-site procedure to directly monitor plant N status in the field throughout the growing season since traditional laboratory tissue testing is inconvenient, time-consuming and cannot be relied upon to provide timely remedial measures for the grower.

A hand-held spectrophotometer, the SPAD-502 chlorophyll meter (Minolta Corporation, Ramsey, N.J.), has increasingly been used to assess the plant N status in crops such as rice (Turner and Jund, 1989; Takebe and Yoneyama, 1989). The SPAD-502 chlorophyll meter allows instant 'real-time', non-destructive measurements of plant leaf greenness, which is correlated to the leaf chlorophyll content (Yadava, 1986; Minolta, 1989). The SPAD-502 chlorophyll meter determines the relative amount of chlorophyll in leaves by measuring the light transmitted through the leaf in two wavelength peaks; a red peak at 650 nm (where absorbance is high and unaffected by carotene) and an infrared peak at 940 nm where absorbance is extremely low (non-chlorophyll absorbance, such as cell walls). After passing through the sample, the quantities of each wavelength are detected by a silicone photodiode and a microprocessor calculates a numerical SPAD unit or value, ranging from 0 to 80, which represents the relative amount of chlorophyll present in the plant leaf. In effect, the SPAD value is proportional to the relative optical density,

based on the ratio between the two wavelengths. The purpose of this study was to evaluate the potential use of the SPAD-502 chlorophyll meter for fast and accurate determination of plant N status in sweet corn and establish critical leaf SPAD values that accurately interpret plant N status, for drip-fertigated N cropping regimes of sweet corn.

Materials and methods

Experimental site: The research was a 2-year field study at Dixon Springs Agricultural Research Center (DSAC), in Simpson, Illinois, USA. Leaf 'SPAD chlorophyll' was monitored over the growing season in response to various rates of applied N fertilizer in two sweet corn cultivars grown under drip-fertigated N culture. The soil at the site was a Grantsburg silt loam, characterized by low to moderate organic matter content (2.2%), with a total N level of 1.16 g kg⁻¹ and a cation exchange capacity (CEC) of 9.7 meq 100 g⁻¹. The site had no previous history of manure application. A pre-plant soil test was performed in spring showed NO₃-N levels at 3.0 mg kg⁻¹.

Treatments and experimental design: Treatments consisted of a factorial combination of two sweet corn cultivars ('Kandy Plus' and 'Sugar Ace') and four rates of N fertilization (0, 84, 168, 336 kg N ha⁻¹). N fertilizer was applied as ammonium nitrate in six applications; one-fifth of the total amount of each N rate was broadcasted as a dry blend pre-plant application and disked-in (0-6 inches). The remaining four-fifths were applied in five applications with the drip-irrigation water using Dosatron DI 16-11 GPM fertilizer injectors (Dosatron International Inc., Clearwater, Florida), beginning 10 days after planting and at 8-10 day intervals thereafter. A split-plot experimental design was used, with N rates as main plots replicated four times and sweet corn cultivars as subplots. Cultivars were machine-seeded in four-row blocks (28 ft rows spaced 2.5 ft apart). Seeds were planted 8-10 inches apart giving a plant population of ~ 64,490 plants/hectare. Data was obtained from plants in the two centre rows. Cultural practices such as cultivation, pest and disease control and maintenance applications of P and K and other cultural requirements were in accordance with standard commercial sweet corn recommendations (Foster *et al.*, 2002).

SPAD measurements: Plant leaves were analyzed at various growth stages throughout the growing seasons for SPAD-chlorophyll and total N concentration. There were four sampling dates in season one, corresponding to the seventh leaf stage (V7), tenth leaf stage (V10), silking (R1), and milking (R3), and three sampling dates in season two (V6, V10, and R1). Leaf SPAD measurements were taken from the uppermost fully expanded leaves (youngest leaves with fully exposed collars) at each vegetative sampling stage and from the ear leaf at each reproductive sampling stage. Eight to ten leaves were harvested from plants in each N x cultivar plot and 20 SPAD readings were taken on the outside 0.5-inch margins of the middle one third of the leaf laminae. This protocol resulted in eight to ten individual leaf SPAD readings for each N x cultivar subplot; readings were averaged over replicates.

Leaf tissue N determination: Total leaf N determination was based on the modified Kjeldahl digestion and the colorimetric assay described by Cataldo *et al.* (1974). Leaf pieces from

the leaves used in the SPAD readings were dried at 80°C and ground to a fine powder in a miniature cyclone mill. Total N was determined in triplicates using a micro-Kjeldahl digestion preceded by a 2.5% salicylic acid: H₂SO₄ (w:v) pre-digestion treatment to facilitate reduction of tissue NO₃ to NH₄ (Nelson and Sommers, 1980). Total N in the digest solution was quantitatively recovered as ammonium-N by colorimetric analysis (625 nm) of an indophenol-blue complex.

Data analysis: Data was analyzed using the SAS statistical package (SAS Institute Inc., Cary N.C.). Factorial data analysis of variance (PROC GLM) with stepwise elimination of non-significant independent variables was used to identify significant variety and N rate fertilizer effects and any N rate x variety interactions at $P \leq 0.05$. Where significant main effects of cultivar were detected, means were separated by the least significant difference procedures (LSD) at $P \leq 0.05$. Trend analysis was used to partition main effects of N fertilization rate into significant linear and quadratic components.

Critical SPAD values were determined in a two-step procedure. First, the optimum N rate (the lowest N fertilizer rate which provided highest marketable ear yield) was determined by SAS trend analysis, using either quadratic regression or quadratic-plateau regression (whichever gave the best fit of the data). This latter form of regression analysis (SAS-NLIN procedure) was chosen over linear or quadratic regression when it resulted in a > 5% reduction in residual error mean square, and at the same time when it provided a better visual explanation of the data. By similar regression techniques, the SPAD readings associated with these N rates were estimated. In the process, relationships between N fertilization rate, leaf SPAD readings, and leaf total N were used to evaluate the effectiveness of the SPAD-chlorophyll 'quick-test' as an on-site predictor of plant N status.

Results

Leaf SPAD: Leaf 'SPAD-chlorophyll' values during both growing seasons showed variations. In season one, leaf SPAD readings were not different among cultivars. However, increasing SPAD values were recorded in both cultivars with advancement in growth stages. The trend was a linear increase early in the season at V7 and V10 stages and a curvilinear increase later in the season; with increase in N rates up to 300 kg N acre⁻¹. Pooled over cultivars and N rates, SPAD values in season one averaged 51.8 at V7, 50.5 at V10, 57.2 at R1 and 48.6 at R3 (Tables 1 and 2).

In season two, leaf SPAD readings were significantly different between the cultivars with 'Sugar Ace' having higher leaf SPAD values than 'Kandy Plus' (Table 2). In each cultivar, N fertilization had a positive linear effect on leaf SPAD readings. Pooled over cultivars and N rates, SPAD values in season two averaged 45.5 at V6, 42.5 at V10 and 50.7 at R1, respectively. In both growing seasons, leaf SPAD readings tended to increase in each cultivar from the early vegetative stage (V7) up to silking (R1). These responses were consistent with daily field observations, which showed distinct differences in leaf greenness between each of the N treatments beginning at V10, and increasing in intensity at R1.

Leaf N levels: The effects of N fertilization rates on leaf N concentrations were significant among cultivars in season two. In this season, leaf N concentrations at V6 and R1 growth stages

were significantly higher in ‘Sugar Ace’ than in ‘Kandy Plus’ (Table 3). Nitrogen fertilization had a positive effect on leaf N concentrations at each sampling date, with leaf N levels increasing curvilinearly with increasing N rate up to 168 kg N ha⁻¹ at V10

Table 1. Effect of cultivar and N fertilization rate on sweet corn leaf ‘SPAD chlorophyll’ readings at various growth stages in season one

Cultivar	Growth stages			
	V7 ^z	V10	R1	R3
‘Kandy Plus’	51.8	50.5	56.9	48.8
‘Sugar Ace’	51.9	50.7	57.4	48.4
Significance	ns	ns	ns	ns
N rate (kg ha ⁻¹)				
0	49.5d	45.6d	51.4c	41.0c
84	51.6c	49.2c	56.2b	48.3b
168	52.6b	52.2b	60.0a	51.7a
336	53.6a	55.1a	61.1a	53.5a
Significance	L***Q*	L***Q*	L***Q***	L***Q***

^zV7 (seventh leaf stage), V10 (tenth leaf stage), R1 (silking), R3 (milk stage ~20 days after silking). Means followed by the same letter in a column are not significantly different.

ns, **, ***, Non-significant, significant at $P \leq 0.05, 0.01, 0.001$, respectively. L (linear) and Q (quadratic) trends.

Table 2. Effect of N fertilizer rates on leaf ‘SPAD-chlorophyll’ readings at three growth stages in two sweet corn cultivars in season two

Cultivar	Growth stages		
	V6 ^z	V10	R1
‘Kandy Plus’	44.1b	41.4b	43.1b
‘Sugar Ace’	46.8a	43.6a	53.2a
Significance	***	**	***
N rate (kg ha ⁻¹)			
0	41.4c	38.6d	45.7c
84	44.7b	41.1c	48.9b
168	46.3b	44.1b	53.2a
336	49.5c	46.2a	54.7a
Significance	L***	L***	L***

^zV6 (sixth leaf stage), V10 (tenth leaf stage), R1 (silking).

Means followed by the same letter in a column are not significantly different.

, *: significant at $P \leq 0.01, 0.001$, respectively. L (linear) trend.

Table 3. Effect of cultivar and N fertilization rate on sweet corn leaf N concentration at various growth stages

Cultivar	N % (dry weight)						
	2001				2002		
	V7 ^z	V10	R1	R3	V6 ^z	V10	R1
‘Kandy Plus’	4.35 ^y	4.09	4.04	3.29	4.01b	2.51	3.10b
‘Sugar Ace’	4.42	4.10	4.03	3.22	4.48a	2.66	3.42a
Significance	NS	NS	NS	NS	***	NS	**
N rate (kg ha ⁻¹)							
0	3.95b	3.56b	3.57d	2.70d	3.85c	2.19c	2.89b
84	4.60a	3.93a	3.85c	3.08c	4.13b	2.47b	3.00b
168	4.34a	4.31a	4.21b	3.50b	4.44a	2.75a	3.55a
336	4.54a	4.59a	4.51a	3.74a	4.56a	2.92a	3.58a
Significance	NS	L***	L***	L***	L***	L***	L***
		Q**	Q**	Q**			

^zV6 (sixth leaf stage), V7 (seventh leaf stage), V10 (Tenth leaf stage), R1 (Silking), R3 (Milk stage ~20 days after silking). Means followed by the same letter in a column are not significantly different. ns, **, ***, Non-significant, significant at $P \leq 0.05, 0.01, 0.001$, respectively. L (linear) and Q (quadratic) trends.

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Table 4. Regression of N rate on leaf SPAD and respective critical SPAD values for three growth stages of sweet corn in season one

Sampling stage	Regression equation	R ²	Critical SPAD ^y
V7	$Y = 49.6 + 0.0286x - 0.000050x^2$	0.72***	52.8
V10	$Y = 45.6 + 0.0548x - 0.000077x^2$	0.82**	52.1
R1	$Y = 51.3 + 0.0807x - 0.000196x^2$	0.87***	59.0

^zData is combined for ‘Kandy Plus’ and ‘Sugar Ace’.

^yBased on an N rate of 155.9 lbs N acre⁻¹.

, * Significant at $P \leq 0.01, 0.001$, respectively.

Table 5. Regression of N rate on leaf SPAD and respective critical SPAD values for three growth stages of sweet corn in season two

Cultivar	Stage	Regression equation	R ²	Critical SPAD ^z
‘Kandy Plus’	V6	$Y = 40.53 + 0.0273x$	0.81***	48.0
	V10	$Y = 39.26 + 0.0164x$	0.48*	43.8
	R1	$Y = 41.6 + 0.082x - 0.000144x^2$	0.95***	53.3
‘Sugar Ace’	V6	$Y = 43.6 + 0.024x$	0.68**	50.3
	V10	$Y = 37.9 + 0.0678x - 0.00011x^2$	0.80**	48.3
	R1	$Y = 50.2 + 0.0228x$	0.67**	56.4

^zBased on an N rate of 273.2 lbs N/acre or 306.2 kg ha⁻¹.

*, **, *** Significant at $P \leq 0.05, 0.01, 0.001$, respectively.

and R3 in season one and linearly at V6, V10 and R1 in season two. Averaged over cultivars and N fertilizer rates, leaf N levels decreased over the growing season, with N concentrations averaging 3.2% at R1 in both years.

Relationship between leaf ‘SPAD-chlorophyll’ and nitrogen:

The relationships between leaf SPAD values and leaf N at V7 (V6), V10, and R1 growth stages in seasons one and two are shown in Figs. 1, 2, and 3, respectively. In season one, there was no significant leaf SPAD and leaf N relationship early in the season; later it developed into a linear relationship at V7 and a curvilinear one at V10. In the second season, the relationships between leaf SPAD and leaf N were largely linear at each growth stage (V6, V10 and R1), indicating that leaf SPAD readings were positively correlated with leaf N. Since the relationship between leaf SPAD and leaf N was linear at all stages it indicated that a large portion of the leaf N in corn leaves is closely associated with chlorophyll.

Critical SPAD values: Critical SPAD values are readings associated with the lowest N fertilizer rate which provided the highest marketable ear yield. In each year, yield data for ‘Kandy Plus’ and ‘Sugar Ace’ were pooled to provide an overall estimate of yield response to N rate. From the response functions generated by the regression of N rate on yield, maximum ear yields were estimated at 13.8 tons ha⁻¹ with 174.8 kg N ha⁻¹ in season one and 9.7 tons ha⁻¹ with 306.2 kg ha⁻¹ in season two. In both years, the quadratic-plateau model was chosen over the quadratic regression because it resulted in a 5% or more reduction in residual error mean square, and at the same time, provided a better visual explanation of the data. By regression techniques, the SPAD readings associated with each of the above optimum N rates were estimated for each growth stage sampled. Additionally, SPAD data in season one were combined for the cultivars to provide an overall estimate of critical SPAD values. However, in season two, due to significant N rate by cultivar interactions affecting leaf SPAD readings at each of the sampling dates, individual sets of critical SPAD values were determined for the cultivars (Table 2). Hence, critical SPAD values in season one were estimated as

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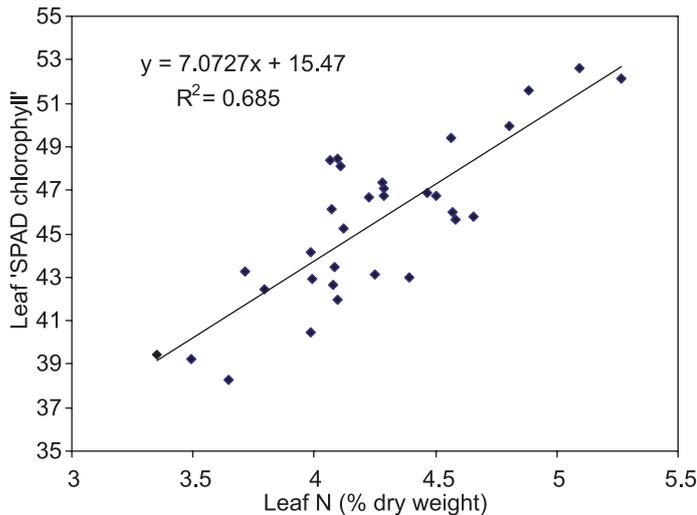


Fig. 1. Relationship between leaf 'SPAD chlorophyll' and leaf N concentration at V6 growth stage in season two

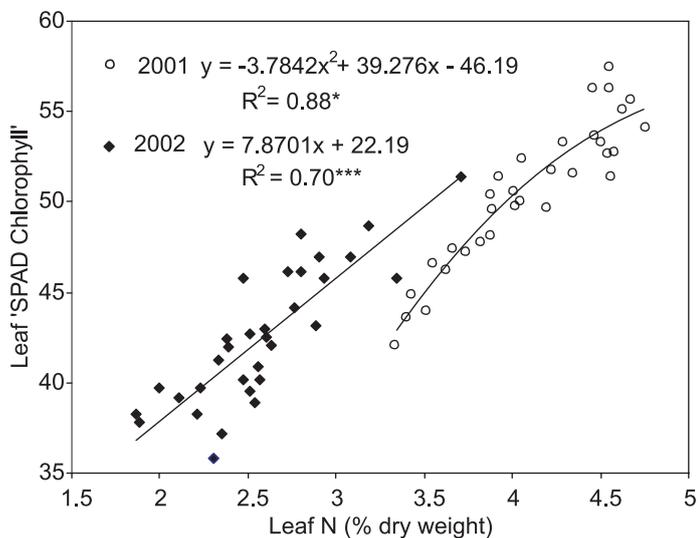


Fig. 2. Relationship between leaf SPAD readings and leaf N concentration at V10 growth stage in the two growing seasons

52.8 at V7, 52.1 at V10 and 59.0 at R1 (Table 4). In the second season, critical SPAD values followed a somewhat similar pattern; increasing from 48.0 at the early sampling date (V6) in 'Kandy Plus' to 56.4 at R1 in 'Sugar Ace' (Table 5). Therefore, the average critical SPAD values pooled over the two years were \approx 49-53 units at V6-7, \approx 46-52 units at V10 and \approx 54-59 units at R1 growth stages.

Discussion

In the present study, leaf 'SPAD chlorophyll' measurements followed a distinct pattern of change over time with different N fertilizer rates, increasing early in the season up to R1 (silking), and declining thereafter at R3 (milk stage or harvesting) and concurrently increasing with increase in N rate. Similar positive correlations between leaf SPAD readings and leaf tissue N concentration have been reported in corn (Sunderman and Lamm, 1991; Schepers *et al.*, 1992; Wood *et al.*, 1992), wheat (Reeves *et al.*, 1993), potatoes (Minotti *et al.*, 1994), strawberry (Himelrick *et al.*, 1993), bell pepper (Hartz *et al.*, 1993), apples (Nielsen *et al.*, 1995) and tomato (Sandoval-Villa *et al.*, 2000). Bullock and Anderson (1998) reported an increase in SPAD values in corn

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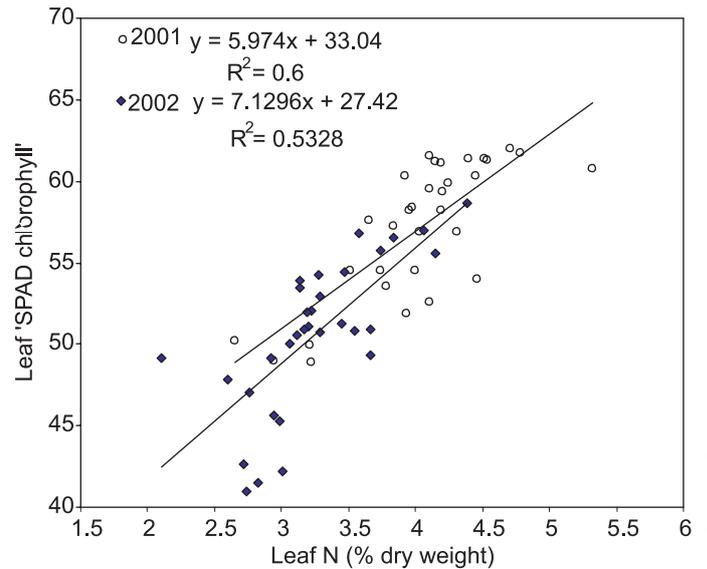


Fig. 3. Relationship between leaf SPAD readings and leaf N concentration at R1 growth stages in the two growing seasons

with plant age from a low at V7, maximum at R1 and low (but higher than at V7) at the R4 growth stage.

The increase in leaf SPAD readings at silking reflects a strong sink/source relationship among the leaves for absorbed N. The strong positive relationship between leaf SPAD readings and leaf N status is largely due to the responsiveness of SPAD-chlorophyll to N fertilizer application. On the basis of these results, it can be concluded that the SPAD meter can be used to effectively assess sweet corn N status early in the season *i.e.* at V10 stage and before silking stage. At these stages it is possible to correct any N deficiencies that might affect yields. Despite the strong linear relationships between the SPAD readings and N rate, and also between SPAD and leaf tissue N concentrations at silking stage (R1) in the evaluation of the crops' N status, it is probably too late in the season to allow correction of in-season N deficiencies at this stage. Previous work on corn indicates that corn N status can be evaluated and effectively corrected at V10 and mid silk stages (Stanley and Rhoads, 1977).

Although the leaf SPAD readings varied significantly with the N-rates, it is difficult to identify specific critical SPAD values when seasons and cultivars are variables, as observed in this study. This calls for a specified range of SPAD values (critical SPAD values) for a specific site within which application of N fertilizer is not necessary. However, there are other underlying factors such as the inherent soil N content and the environment, which have to be taken to account. The fairly uniform critical SPAD readings at the maximum marketable yields indicate that there is a strong relationship between the marketable yield and leaf SPAD readings especially early in the season, at V7 and V10 growth stages. This observation also shows promise for utilization of the SPAD meter in prediction of sweet corn N status early in the growing season. The range of critical SPAD values can be considered as benchmarks above which no further addition of N would be necessary. Minotti *et al.* (1994) estimated threshold SPAD values for potatoes, which are the same as critical SPAD values within a range from \approx 49-56 units; these were considered as tentative benchmarks above which no further addition of N was

necessary. However, in the present study, the lack of consistency between the years with respect to the maximum N rate depicts variations between seasons. That notwithstanding, leaf SPAD readings can be given as ranges within which it is possible to correct any moderate in-season nitrogen deficiencies.

Results from this study have shown that there are unique inherent differences between cultivars with regard to leaf greenness and leaf N concentration and yield potential. Generally, 'Sugar Ace' cultivar consistently had higher leaf SPAD values and leaf N concentration at each growth stage and N fertilizer rate compared to 'Kandy Plus'. However, these differences may not be too critical to limit the use of the SPAD meter across cultivars, especially within a range of critical SPAD values to determine the plant N status in sweet corn. Significant cultivar effects on the SPAD readings has also been noted in potatoes (Minotti *et al.*, 1994), maize (Schepers *et al.*, 1992), apples (Neilsen *et al.*, 1995) and tomatoes (Sandoval-Villa *et al.*, 2000).

Diagnosis of sweet corn N status using the SPAD meter may be limited by the fact that a large portion of the leaf N is in the form of NO_3^- which is not associated with the chlorophyll molecule, as suggested by Takebe and Yoneyama (1989). In studies conducted on rice, little NO_3^- nitrogen was found in the leaves at any stage of growth, even though there were highly significant and positive correlations between the SPAD readings and the leaf N concentrations (Takebe and Yoneyama, 1989).

In conclusion, the significant relationship between leaf SPAD and N rate shows promise for the utilization of the SPAD-502 meter in predicting sweet corn N status across seasons and cultivars, especially at V10 stage. Determination of the plant N status using the SPAD-502 meter is rapid, convenient, reliable and non-destructive to leaf tissue and could therefore be a viable, on-farm technique to supplement conventional laboratory tissue testing in assessing plant N-status.

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