USE OF CHLOROPHYLL FLUORESCENCE TECHNIQUES TO DETECT STRESSES IN CORN

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Abstract

Increased efficiencies in the use of water and fertilizer will require better methods of monitoring crop stress. This study was conducted to determine whether chlorophyll fluorescence was more sensitive to detecting water and nitrogen stress than chlorophyll meters in corn (*Zea mays*). The experiment was carried out near Shelton, NE in 2000, 2001, and 2002. Treatments consisted of a factorial combination of 12 corn hybrids (11 Pioneer Hi-Bred International[1] and B73 x MO17), two water levels (deficit and full irrigation), and two N levels (starter fertilizer only and full N), for a total of 48 treatments combinations, with three replications. Leaf chlorophyll content was estimated with a Minolta 502 SPAD chlorophyll meter. A Walz PAM-2000 Portable Chlorophyll Fluorometer[1] was used to measure leaf temperature. thylakoid electron transport rate (ETR), and the ratio of the variable fluorescence signal to the maximum fluorescence signal (Fv/Fm). Measurements were taken in late vegetative growth stages and throughout the reproductive stages. The data thus far analyzed indicated higher ETR and SPAD values for water unstressed plots while[1] water stressed plots had higher leaf temperatures. Chlorophyll fluorescence can be used as an effective tool to detect drought and N stress in corn.

Introduction

Chlorophyll (CHL) is the major pigment associated with harvesting of solar energy by plants, with light energy being converted to photochemical energy used in the assimilation of CO_2. Chlorophyll fluorescence measures the efficiency of the light harvesting processes associated with photo-system II. Fluorescence measurements have been shown to be very sensitive (pre-visual) indicators of various plant stresses such as water, nutrients, temperature, etc. This technology is relatively new and in recent years has become much easier to utilize for field measurements. The patented pulse amplitude modulation (PAM) principle (WALZ model PAM-2000 Portable Chlorophyll Fluorometer) was used in our work to measure fluorescence parameters in field grown corn. The leaf clip measuring apparatus attached to a leaf simultaneously acquired chlorophyll fluorescence, leaf temperature, and photosynthetic photon flux density (PPFD) on light-adapted leaves. Earl and Tollenaar (1999) have shown that variation in thylakoid electron transport rate as determined with PAM fluorometry is associated with photosynthetic performance of maize hybrids under field conditions. Greater Fv/Fm values indicate better functionality of the photosynthetic apparatus (Lichtenthaler and Rinderle, 1988). The objective of this study was to evaluate the use of chlorophyll fluorescence techniques versus the SPAD chlorophyll meter as a means of detecting crop N and water stresses and the resultant impact on grain yield.

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[1] Trade and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product by the authors or USDA.

Methods

The work was conducted near Shelton, NE during the 2000, 2001, and 2002 growing seasons. The experimental design was a split-split-split plot design, with water as the main plot, N as the split-split plot, and hybrid as the split-split-split plot. Individual plot dimensions were 3.6 x 30 m, consisting of four rows spaced 0.9 m apart. Leaf chlorophyll content was determined with a Minolta 502 SPAD chlorophyll meter on the midpoint between the collar and leaf tip. and halfway between the leaf margin the mid rib on the uppermost fully expanded leaf or the ear leaf, if discernable, on 30 plants per plot and the values were averaged for each plot. Chlorophyll fluorescence parameters and leaf temperature were measured simultaneously with a Walz PAM-2000 portable chlorophyll fluorometer.

Chlorophyll fluorescence was measured on both light or dark-adapted plant material with slightly different procedures. Light-adapted measurements were measured on the same area of the leaf as the SPAD readings, but care was taken to select leaf tissue that was well illuminated. Fifteen plants were measured from each plot and a plot average was calculated. The light-adapted output parameters from the PAM-2000 were: Photochemical quantum yield (Fv/Fm'), photosynthetic photon flux density (PPFD), and leaf temperature (determined with thermocouple located on leaf clip). Electron transport rate (ETR) was calculated as: ETR=(PPFD x Fv/Fm' x Leaf absorptance x 0.4) according to Earl and Tollenaar (1999), where leaf absorptance was determined from SPAD measurements as per Earl and Tollenaar (1999). Light-adapted measurements were taken on three dates in 2000 (Julian days 188, 213, and 223), two dates in 2001 (Julian days 212 and 226) and on three dates in 2002 (Julian days 196, 218, and 227). In 2001 and 2002, only the full N plots were measured. Specially made leaf clips were made to clip onto a leaf to completely shade a 2 cm^2 area. These were left on the leaf for at least 20 minutes (Lichtenthaler and Rinderle). The dark-adapted output parameters from the PAM-2000 were Fo, which is the minimum level of fluorescence for dark-adapted plat tissue, Fm, which is the maximum level of fluorescence while a saturating pulse of white light is applied, and Fv/Fm. Fv/Fm is the ratio of the variable fluorescence (Fm - Fo), Fv to Fm. Dark-adapted measurements were taken on two dates in 2001 (Julian days 214 and 221) on five hybrids with both water treatments and full N, with three measurements taken from each plot and averaged within a plot. In 2002, dark-adapted measurements were taken on all 12 hybrids under both water levels and full N. Ten measurements were taken for each plot and averaged within a plot on Julian day 226.

Final grain yield (adjusted to constant moisture) at maturity was determined by machine harvest (2002 grain yield data have yet to be analyzed). The analysis of variance (ANOVA) and linear correlation analysis were used to determine treatment effects and associations among measured variables.

Results

The ANOVA of the 2000 data revealed significant water, N, and hybrid main effects as well as interactions among factors for grain yield, with water and N application increasing grain yields and average of 38% and 116%, respectively (not shown). The ANOVA also revealed significant water, N, and hybrid main effects as well as interactions among factors for SPAD, leaf temperature, and ETR values across all three dates (Table 2).

Leaf temperature and ETR values, as determined with fluorometer measurements, indicated the presence of a water stress as early as the second date, with leaf temperatures increased and ETR values decreased for the water stressed treatment relative to the full irrigation treatment (Fig. 1). These differences were likely due to reduced transpirational cooling of the canopy, which resulted in elevated leaf temperatures and sub-optimal conditions for leaf photosynthesis. Water stress affected SPAD values only on the final observation date (Fig. 1), with water stress decreasing SPAD values on this date relative to the non-stressed water treatment. This was likely due to the effect the prolonged water stress has on chlorophyll degradation. Differences in ETR and leaf temperature water treatment means were observed much earlier than differences in the water treatment means of SPAD values, with ETR reduced and leaf temperatures increased by water stress (Fig. 1), indicating that fluorescence assessments were more sensitive than SPAD measurements in detecting water stress.

Nitrogen stress drastically reduced SPAD and ETR values and increased leaf temperature on all measurement dates (Fig. 2), indicating that the N treatment produced drastic modifications in plant productivity potential.

Linear correlation analysis was used to determine the association between treatment variation in grain yield and the other independent variables on the 2000 dates (Tables 1,2, and 3). Grain yield was more highly correlated with SPAD readings than ETR on the early measuring date, which corresponded approximately to the V-9 growth stage of the crop (Table 1). However, on the later date, which corresponded to the R-4 growth stage, grain yield was more highly correlated with ETR values than SPAD values (Table 2). Additionally, leaf temperature values were negatively associated with grain yield and ETR values, further illustrating the negative effects of water stress present on this date (Table 2). ETR and leaf temperature assessments collected with the PAM-2000 were more sensitive than SPAD in detecting stress.

Data from the 2001 and 2002 growing seasons are still being summarized.

Summary

The chlorophyll fluorescence technology utilized in our work was more sensitive than the SPAD chlorophyll meter in detecting water stress in corn, while both technologies were equally capable of detecting the more obvious N stress generated in this experiment.

References

Earl, H. J. and M. Tollenaar. 1999. Using chlorophyll fluorometry to compare photosynthetic performance of commercial maize (Zea mays L.) hybrids in the field. Field Crops Res. 61:201-210.

Lichtenthaler, H.K. and U. Rinderle. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytical Chemistry. 19(1):S29-S85

Tables and Figures

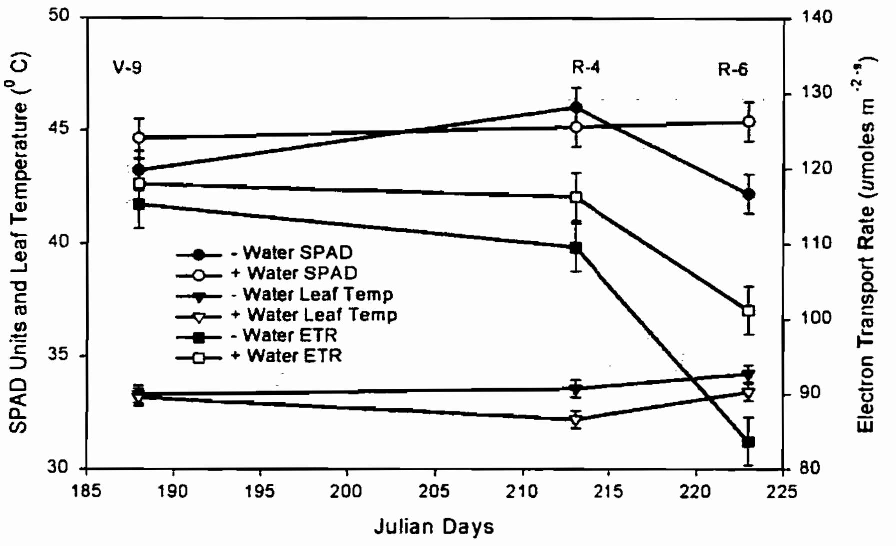

Fig. 1. Response of leaf SPAD, temperature, and ETR values to the main effects of irrigation treatments across year 2000 calendar dates.

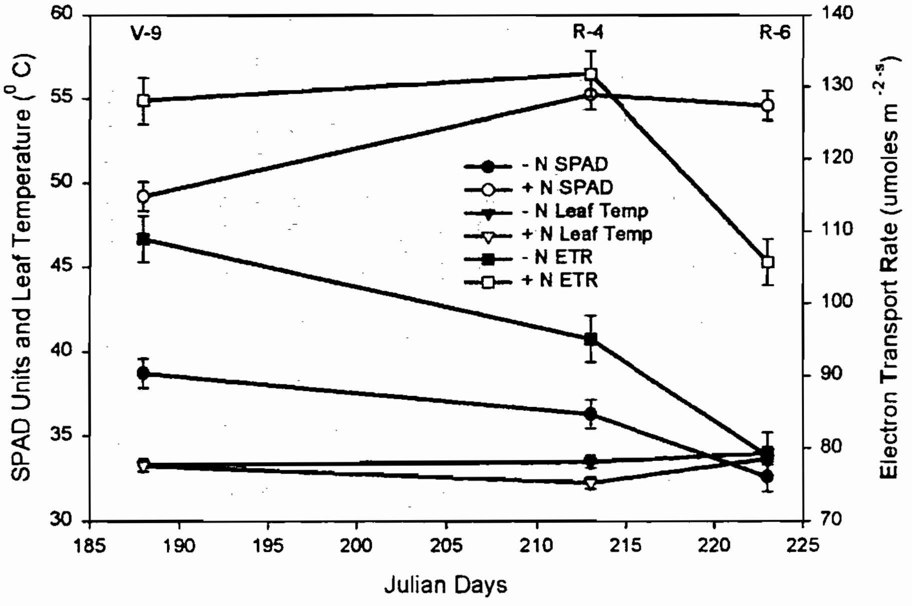

Fig. 2. Response of leaf SPAD, temperature, and ETR values to the main effects of N treatments across year 2000 calendar dates.

Table 1. Correlation coefficients among grain yield and leaf physiological variables for Julian Day 188.

	Grain Yield	SPAD	ETR	Leaf Temperature
Grain Yield	1			
SPAD	0.895**	1		
ETR	0.678**	0.719**	1	
Leaf Tmp	-0.147	-0.086	-0.415	1

Table 2. Correlation coefficients among grain yield and leaf physiological variables for Julian Day 213.

	Grain Yield	SPAD	ETR	Leaf Temperature
Grain Yield	1			
SPAD	0.838**	1		
ETR	0.878**	0.842**	1	
Leaf Temperature	-0.771**	-0.533**	-0.721**	1

Table 3. Correlation coefficients among grain yield and leaf physiological variables for Julian Day 223.

	Grain Yield	SPAD	JE1200	Leaf Temperature
Grain Yield	1			
SPAD	0.919**	1		
JE1200	0.853**	0.740**	1	
Leaf Temperature	-0.114	-0.01192	-0.35674	1

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