Characterizing the major U.S. maize heterotic groups by linking SPAD values, N use traits, and grain yield

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Introduction:

- Our research has shown that the various U.S. maize HGs have diverse genetic tendencies in how yield is achieved through either kernel number or kernel weight.
- Yield at Low N is mainly determined by kernel number and yield response to added N is highly influenced by kernel weight.
- These two yield component traits are inversely related and are primarily influenced by the N use traits NUt and NUp, respectively.
- NUE is a composite trait of NUt and Nup, and is viewed as the yield response per unit of added N.
- Whole plant sampling and processing is needed to determine NUt and NUp, and has a high demand on time and resources.
- The SPAD chlorophyll meter is a reliable tool to quantify in-season leaf N concentration.
- The use of this device as a non-destructive, in-season measurement of leaf N concentration in response to N availability could be used as a proxy for NUt and/or NUp and be used to predict hybrid NUE to eliminate the need for whole plant sampling.

Materials and Methods:

- This experiment was conducted during 2011 and 2012 at Champaign-Urbana, IL. The soil type was a Drummer-Flanagan soil association (Typic Endoaquolls).
- 10 ex-PVP inbred lines, B73, and Mo17 were combined in a factorial pattern (Table 1). Historic mating patterns were maintained. This resulted in 4 females X 8 males for 32 F_1 hybrids.

Table 1: Germplasm entries that span the genetic diversity of current US maize hybrids.

Type	Inbred	Patentee	Heterotic Group (HG)
Female	B73	None (Public)	SSS
	LH1	Holden's Foundation Seed	SSS
	PHG39	Pioneer Hi-Bred International	SSS/Amargo/Iodent
	PHJ40	Pioneer Hi-Bred International	SSS/Minn13
Male	LH123ht	Holden's Foundation Seed	Pioneer Hybrid 3535/Lancaster
	LH82	Holden's Foundation Seed	Pioneer Hybrid 3558/Minn13
	Mo17	None (Public)	Lancaster
	PH207	Pioneer Hi-Bred International	Iodent
	PHG35	Pioneer Hi-Bred International	Oh07-Midland/Iodent
	PHG47	Pioneer Hi-Bred International	Oh43
	PHG84	Pioneer Hi-Bred International	Oh07-Midland/848
	PHZ51	Pioneer Hi-Bred International	848

- Two N fertilizer rates were used (0 and 252 kg N ha^{-1}) to characterize hybrid low N tolerance and maximum N response.
- SPAD measurements were collected for 10 random plants in each plot and were averaged for the final SPAD value.
- Measurements were taken at the physiological growth stages V10, R1, and during grain fill (GF).
- Calculation of NUp, NUt, and NUE:

$$NUp = Plant\ N$$

$$NUt = \frac{(Yield)}{(Plant\ N)}$$

$$NUE = \frac{(Yield_{+N} - Yield_{-N})}{(N\ rate)}$$

Question: Can SPAD values be used as a proxy for N utilization (NUt) and/or N uptake (NUp) in the prediction of N use efficiency (NUE) of maize heterotic groups (HG) and hybrids?

Growth Stage SPAD Value Correlation With Grain Yield:

Table 2: Correlations between grain yield and the three SPAD growth stage timings within the Low and High N rates.*

		Yield	SPAD at V10	SPAD at R1	SPAD at GF
High N	Yield		0.14 *0.0566*	0.25 *0.0004*	0.36 *<0.0001*
	SPAD at V10	0.61 *<0.0001*		0.64 *<0.0001*	0.49 *<0.0001*
	SPAD at R1	0.58 *<0.0001*	0.78 *<0.0001*		0.65 *<0.0001*
	SPAD at GF	0.67 *<0.0001*	0.78 *<0.0001*	0.79 *<0.0001*	
Low N					

*The top value is the Pearson correlation coefficient and the italicized value is the p-value of the correlation.

- Yield at Low N was weakly correlated to the SPAD values when compared to the same correlations at High N.
- This may indicate that SPAD values are more predictive of yield in high N environments due to the partitioning of chlorophyll N in the leaves.
- For brevity, only the R1 SPAD timing will be discussed because of the relationship that this growth stage has to the determination of sink availability and yield.

Average Hybrid SPAD Value Within the Parental HG's:

Table 3: The average R1 SPAD value for the parental HG representatives at Low N.

Tukey Grouping	SPAD Mean*	N	Female Parent
A	44.4	48	PHJ40
A B	43.3	48	LH1
B	42.1	48	B73
C	40.4	48	PHG39

LSD = 1.6

Tukey Grouping	SPAD Mean*	N	Male Parent
A	45.7	24	PH207
A B	45.2	24	PHG47
A B	44.2	24	PHG35
B C	42.8	24	Mo17
C D	41.4	24	PHZ51
C D	40.8	24	LH123HT
C D	40.4	24	LH82
D	40.0	24	PHG84

LSD = 2.7

*Values are an indication of the relative amount of chlorophyll present in the leaves.

- Significant SPAD value differences were found between the HGs at both N levels indicating genetic differences in leaf N partitioning.
- Interestingly, there were minor changes in rank between the two N rates, so only one N environment may be needed for SPAD characterization.

Table 4: The average R1 SPAD value for the parental HG representatives at High N.

Tukey Grouping	SPAD Mean*	N	Female Parent
A	54.0	48	LH1
A B	53.5	48	PHJ40
B C	52.4	48	B73
C	51.4	48	PHG39

LSD = 1.5

Tukey Grouping	SPAD Mean*	N	Male Parent
A	57.3	24	PH207
A	56.3	24	PHG47
B	53.2	24	PHG35
B	52.6	24	Mo17
B	52.5	24	PHG84
B C	51.3	24	LH123HT
C	49.9	24	PHZ51
C	49.7	24	LH82

LSD = 2.5

*Values are an indication of the relative amount of chlorophyll present in the leaves.

Average Hybrid NUt Value Within the Parental HG's:

Table 5: The average NUt value for the parental HG representatives at Low N.

Tukey Grouping	NUt Mean*	N	Female Parent
A	51.4	48	PHJ40
A	50.9	48	B73
A B	48.5	48	LH1
B	43.8	48	PHG39

LSD = 5.1

Tukey Grouping	NUt Mean*	N	Male Parent
A	57.2	24	PHG47
A B	55.0	24	LH82
A B C	50.5	24	PH207
B C	48.3	24	PHZ51
C	46.3	24	Mo17
C	46.1	24	PHG84
C	43.2	24	LH123HT
C	42.5	24	PHG35

LSD = 8.6

*Kg ha^{-1} grain per Kg ha^{-1} plant N content.

- The NUt values decreased with greater N availability due to the diminishing yield returns per unit of added plant N.
- Significant NUt value differences between the HGs suggests that there is genetic variation for the amount of yield gained per unit of plant N.

Table 6: The average NUt value for the parental HG representatives at High N.

Tukey Grouping	NUt Mean*	N	Female Parent
A	45.2	48	PHJ40
A B	42.2	48	B73
B C	39.1	48	LH1
C	37.7	48	PHG39

LSD = 3.9

Tukey Grouping	NUt Mean*	N	Male Parent
A	47.4	24	LH82
A B	45.1	24	PHG47
A B C	42.4	24	PHZ51
B C	40.6	24	Mo17
B C	39.9	24	PH207
C	38.4	24	PHG84
C	37.8	24	PHG35
C	36.5	24	LH123HT

LSD = 6.5

*Kg ha^{-1} grain per Kg ha^{-1} plant N content.

Average Hybrid NUp Value Within the Parental HG's:

Table 7: The average NUp value for the parental HG representatives at Low N.

Tukey Grouping	NUp Mean*	N	Female Parent
A	101.7	48	PHG39
A B	98.2	48	B73
B C	88.5	48	PHJ40
C	85.9	48	LH1

LSD = 11.6

Tukey Grouping	NUp Mean*	N	Male Parent
A	102.3	24	PHZ51
A	96.7	24	PHG47
A	96.5	24	PHG35
A	92.9	24	PH207
A	92.0	24	LH82
A	90.0	24	Mo17
A	89.7	24	PHG84
A	88.5	24	LH123HT

LSD = 19.5

*Kg ha^{-1} plant N content.

- The NUp values increased with greater N availability due to the greater amounts of available N.
- Significant NUp value differences show that the HGs differ in their ability to take up available N.

Table 8: The average NUp value for the parental HG representatives at High N.

Tukey Grouping	NUp Mean*	N	Female Parent
A	178.7	48	PHG39
B	160.2	48	B73
B	155.5	48	LH1
C	138.7	48	PHJ40

LSD = 12.0

Tukey Grouping	NUp Mean*	N	Male Parent
A	167.8	24	LH123HT
A B	165.2	24	PHZ51
A B	164.0	24	PH207
A B	163.4	24	PHG84
A B	156.2	24	PHG35
A B	155.3	24	PHG47
A B	148.6	24	LH82
B	145.6	24	Mo17

LSD = 20.1

*Kg ha^{-1} plant N content.

Correlating SPAD Values and Yield With NUp, NUt, and NUE:

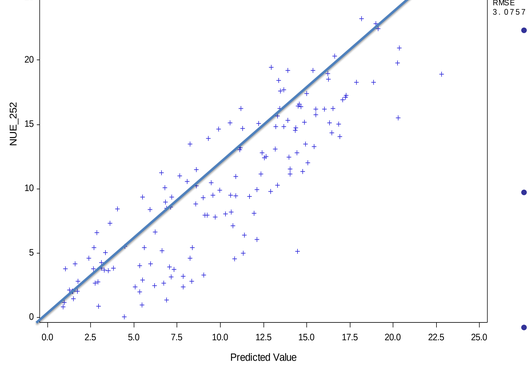
Table 9: Correlations between SPAD and yield at Low and High N vs. the N use traits.*

		NUt	NUp	
SPAD at R1	Low N	-0.23 *0.0014*	0.45 *<0.0001*	NUE
	High N	-0.01 *0.9089*	0.60 *<0.0001*	0.60 *<0.0001*
Yield	Low N	0.46 *<0.0001*	0.42 *<0.0001*	NUE
	High N	0.42 *<0.0001*	0.66 *<0.0001*	0.84 *<0.0001*

* The top value is the Pearson correlation coefficient and the italicized number is the p-value of the correlation.

- The correlations at Low N between NUt, SPAD values, and yield suggests that hybrids sequester greater amounts of N to the grain resulting in less kernel abortion.
- NUp at High N was highly correlated with SPAD values and yield, suggesting that the extra N acquired was sequestered mainly to the leaves.
- NUE was highly correlated with yield and it appears that NUE is mainly determined by NUp.
- The relationship between SPAD values, NUp, and NUE could assist breeders in breeding for hybrids that take up more N, have a greater yield response to applied N, and lose the need for multiple N environments and whole plant sampling.

Predicting NUE Values For Novel Hybrids:



Parameter Estimate Table

Variable	Regression Estimate	Error	P value
Intercept	-24.07974	2.59540	<0.0001
Yield	3.39182	0.19420	<0.0001
SPAD at R1	0.19743	0.05157	0.0002

Figure 1: Linear regression was conducted using the hybrid yield and R1 SPAD value to predict hybrid NUE. The y-axis is the actual NUE and the x-axis is the predicted NUE using the regression model.

- A regression model was created to predict hybrid NUE to eliminate the need for multiple N environments and whole plant processing for N concentration.
- Yield at High N was used in the model due to its high correlation with NUE and the fact that yield is the end goal of any successful breeding program.
- SPAD values at R1 were used as a proxy for NUp and viewed as the hybrid's response to additional N which in turn helps determine the hybrids yield response.
- Although the predicted NUE values are lower than the actual NUE values, the general rankings of the hybrids are conserved leading to the conclusion that using these variables in a predictive model results in high predictive accuracy for hybrid NUE.

Conclusions:

- Significant differences in average SPAD value, NUt, and NUp were detected across the HGs at both N levels, indicating a genetic basis for differing N use traits in maize and that HG ranks were fairly conserved over N rate (Table 3 & 4, 5 & 6, and 7 & 8, respectively).
- At Low N, high yielding hybrids have high NUt and N is preferentially deposited into the grain which reduces kernel abortion (Table 9).
- At High N, high yielding hybrids have high NUp which increases the leaf chlorophyll concentration. This can be viewed as an increase in photosynthetic capacity and grain fill potential (Table 9).
- NUE was highly correlated with SPAD value and yield at High N, and using these variables in a predictive model for NUE showed great promise (Table 9 and Figure 1).
- Breeding for improved hybrid NUE is an expensive and laborious process, so using physiologically based predictive models to reduce this burden will open the doors to greater breeding interest and faster NUE gains in maize hybrids.