



Introduction

- Over time, increases in maize hybrid grain yield have been accompanied with changes in gross morphological traits.

Traits exhibited by modern maize hybrids (Duvick et al., 2004)	
Small Tassels	Short Anthesis-Silking Interval (ASI)
Increased Grain Starch	Higher Staygreen Score
Upright Leaves	Less Lodging
Fewer Tillers	Fewer Barren Plants

- Field spectrometers allow for monitoring of leaf traits like relative water and chlorophyll concentrations throughout the growing season.
- Chlorophyll concentrations serve as a proxy for crop gross primary production (Peng et al., 2011).

Objectives

- Measure leaf chlorophyll content and water content nondestructively using a spectrometer.
- Analyze genetic gain for these and other agronomic traits.
- Identify specific points in the growing season when genetic gains are exaggerated.

Materials and Methods

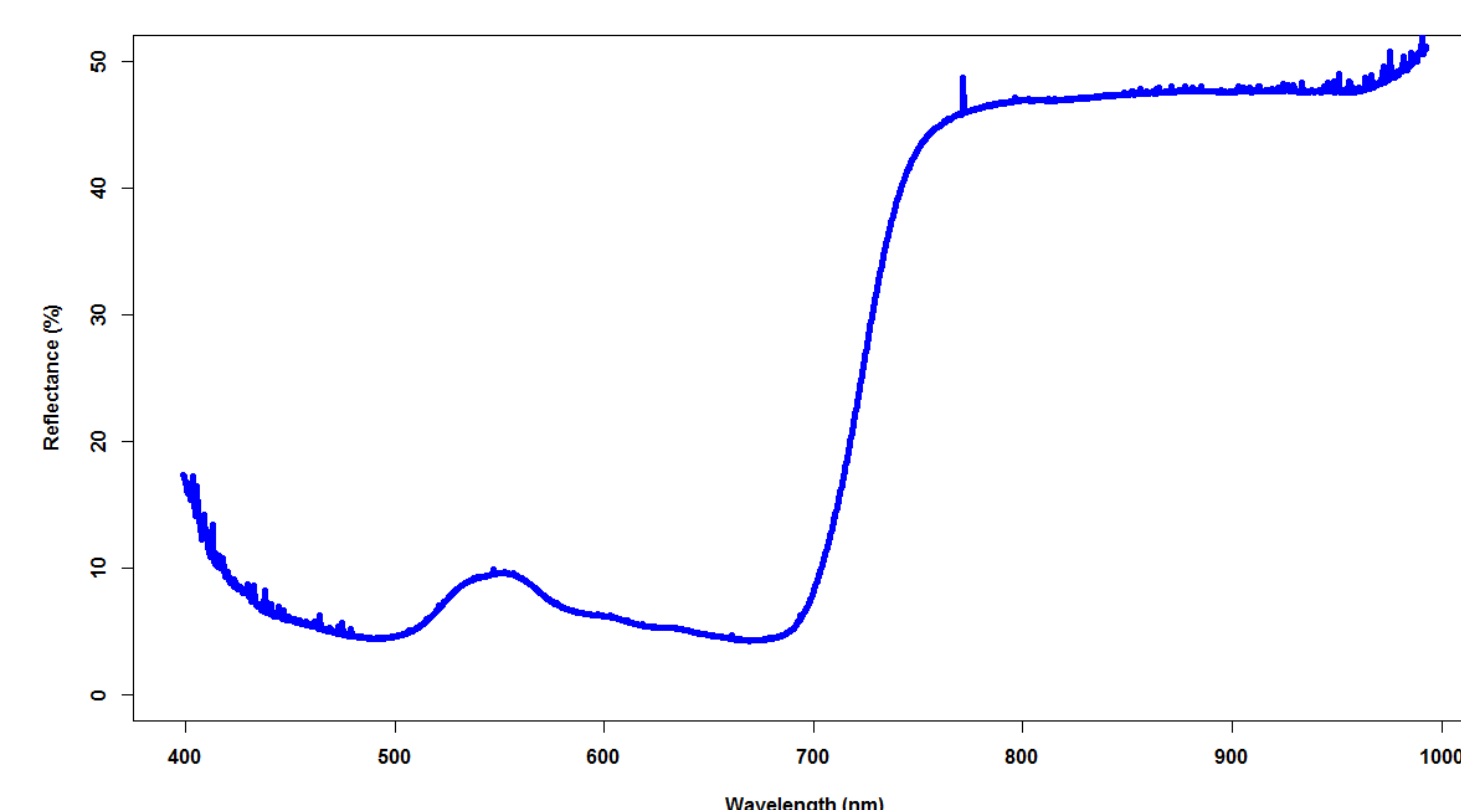
- 36 popular commercial hybrids released from 1936 to 2012.
- Evaluated in two-row plots in Lincoln, NE (2014 and 2015).
- Randomized complete block design with two treatments: irrigated (WW) and rain-fed (WS). Three replications per treatment.
 - Due to excessive rainfall in 2015 both treatments were considered well-watered and the WS treatment from 2014 was discarded from all analysis except for the correlations.
- Phenotypes collected:

Trait	Method
Flowering Dates	50% Anthesis and Silking
Biomass Estimation	Nondestructive Measurements
Canopy Temperature	Infrared Thermometer
Relative Water Content	$[(FW-DW) / (TW-DW)] * 100$
Chlorophyll Content	Microplate Reader
Reflectance Indices	Ocean Optics Spectrometer
Grain Yield, Lodging, Plant Height, Senescence	

Pedigree	Year of Release
307HYB	1936
WF9/38-11//Hy/L317	1948
NS 0	1948
WF9/Hy//L289/1205	1950
329HYB	1954
W64A/OH43	1954
B37/B14//C103/OH43	1958
B14A/B57	1963
N501D	1964
B37/OH43	1965
B37/B14//Mo17	1965
3390	1967
3334	1969
N7A/Mo17	1970
3366	1972
NS[RFS_NB]3_8	1972
B73/Mo17	1974
3541	1975
B73/LH39	1982
B73/LH51	1983
LH132/LH51	1985
LH156/MBS2333	1988
LH132/LH59	1988
3379	1988
LH192 /LH82	1991
3394	1991
33A14	1997
33P67	1999
2A555	2007
33D49	2008
H-7949	2010
7630RR	2011
P0876HR	2012
P0987HR	2012
7644 Hx/LL/RR	2012
N45P-4011	2012



Left: List of era hybrids.
Above: Spectrometer use in the field.
Below: Typical reflectance signature of a maize leaf.



Correlations

On a plot by plot basis, two indices correlated strongly with both chlorophyll and water content: (760/730) (Winterhalter et al., 2011) and Chlorophyll Index Red Edge (Cl_{RE}) (Gitelson et al., 2005).

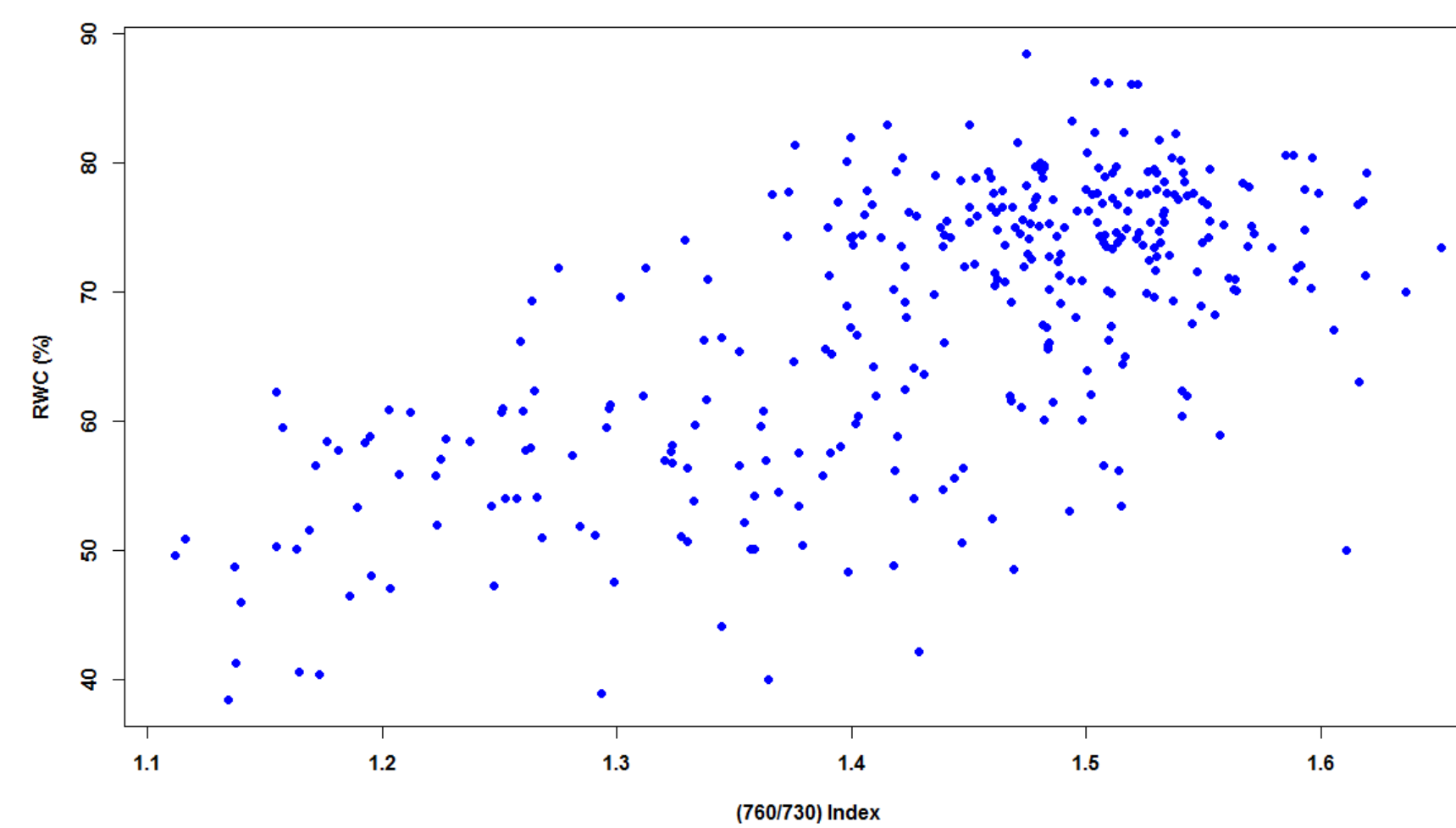


Figure 1. Scatterplot of (760/730) index and relative water content.

Table 1. Coefficients of determination for reflectance indices with chlorophyll (Chl) and water content (RWC).

	Chl	RWC
(760/730)	0.65	0.43
Cl_{RE}	0.62	0.43

Leaf Traits

ANOVA results indicate that the indices as well as the measured chlorophyll and water contents can detect differences among all hybrids.

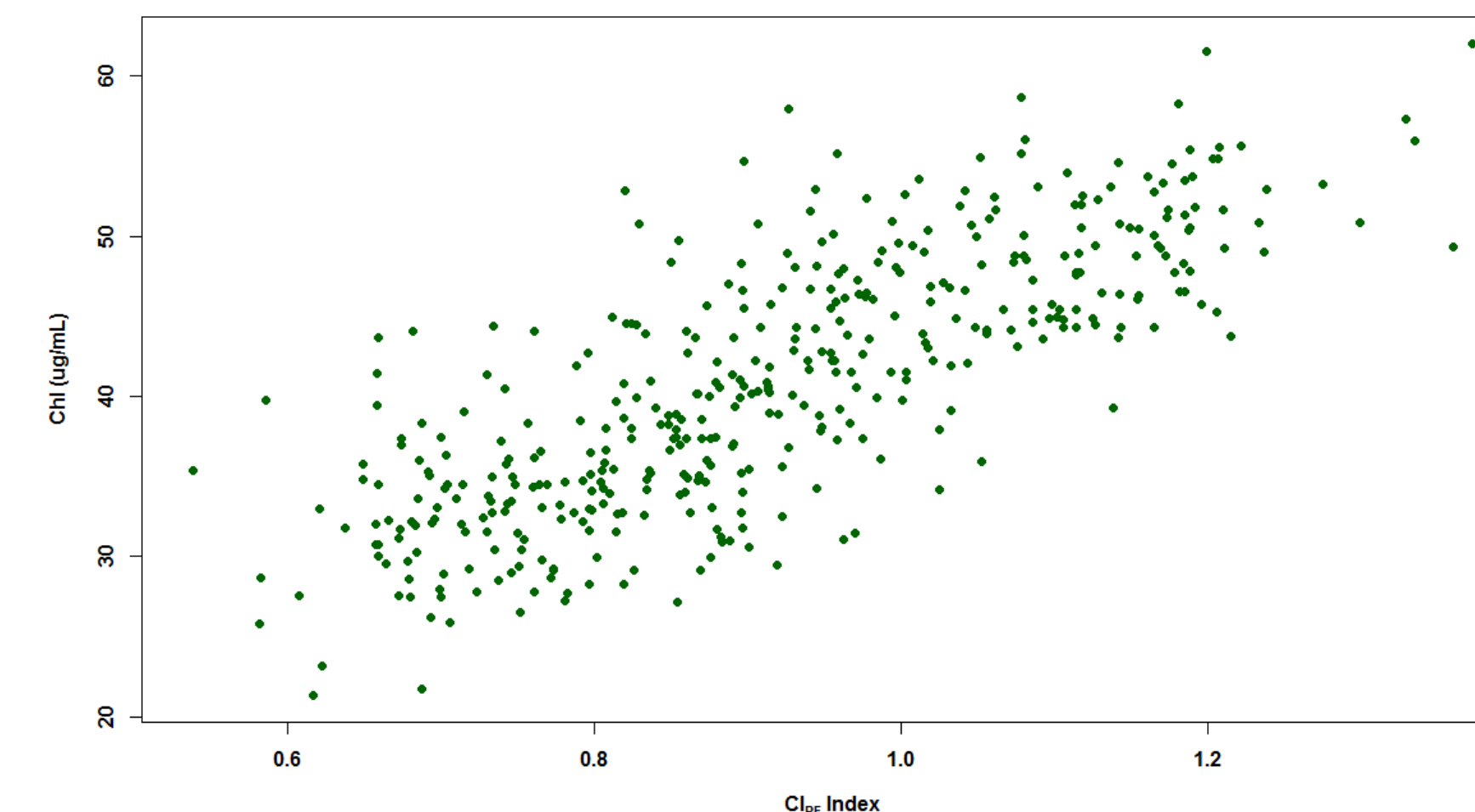


Figure 2. Scatterplot of Cl_{RE} index and chlorophyll concentration.

Source	df	14-Jul-15				7-Aug-15			
		Chl	RWC	(760/730)	Cl_{RE}	Chl	RWC	(760/730)	Cl_{RE}
		MS	MS	MS	MS	MS	MS	MS	MS
Hybrid	35	46.76***	6.31**	0.005***	0.026***	83.55***	6.1***	0.009***	0.053***
Rep	5	94.01***	18.79***	0.001	0.003	63.05**	19.13***	0.005**	0.036**
Error	175	16.07	3.05	0.001	0.007	15.53	2.25	0.001	0.009
Mean		35.83	93.28	1.38	0.835	46.62	91.33	1.42	0.911
CV		0.109	0.019	0.026	0.099	0.086	0.016	0.026	0.098
Heritability		0.608	0.453	0.732	0.732	0.803	0.554	0.832	0.816

Table 2. ANOVA results for chlorophyll content (Chl), relative water content (RWC), and the two indices ((760/730) and Cl_{RE}) for two sampling dates during 2015.

Although chlorophyll and water contents were only measured twice per year for the correlation analysis, the spectrometer took nondestructive measurements frequently throughout the growing season. From the previous results, it is obvious that the indices provide a robust indicator of chlorophyll content.

Genetic Gain of Agronomic Traits

Linear regression models were fit to analyze how traits have changed in maize hybrids over time. In agreement with Duvick, et al. (2004) modern hybrids exhibit a short ASI, lower ear height, less root lodging, and decreased senescence. In addition, at certain points in the growing season, modern hybrids showed increased water and chlorophyll contents.

Table 3. The significance of the p-value, the slope (b), and the coefficient of determination (R^2) from linear regression models fit for each trait over hybrid year of release.

Trait	P-value	b	R^2
Grain Yield	***	0.076	0.709
ASI	***	-0.065	0.633
Plant Height	*	-0.233	0.233
Ear Height	***	-0.362	0.457
Root Lodge (%)	***	-0.004	0.488
Stalk Lodge (%)	***	-0.001	0.540
RWC (R3)	***	0.022	0.376
Chl (R3)	**	0.090	0.357
Senescence	**	-0.041	0.312

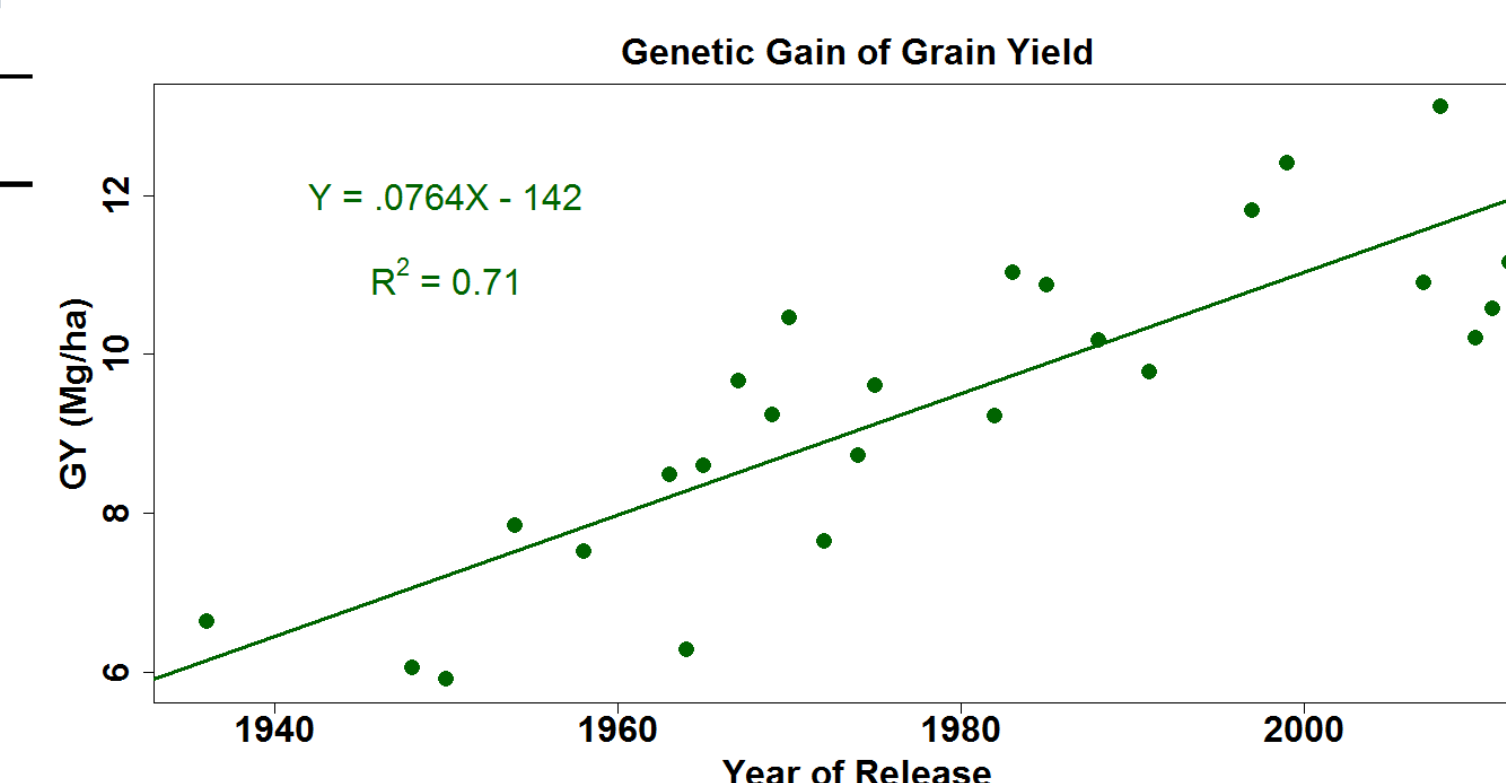


Figure 3. Linear regression of grain yield over year of hybrid release.

Results and Discussion

Power of Reflectance Indices

The (760/730) index shows that it can differentiate old and new hybrids more robustly than laboratory measurements of chlorophyll content.

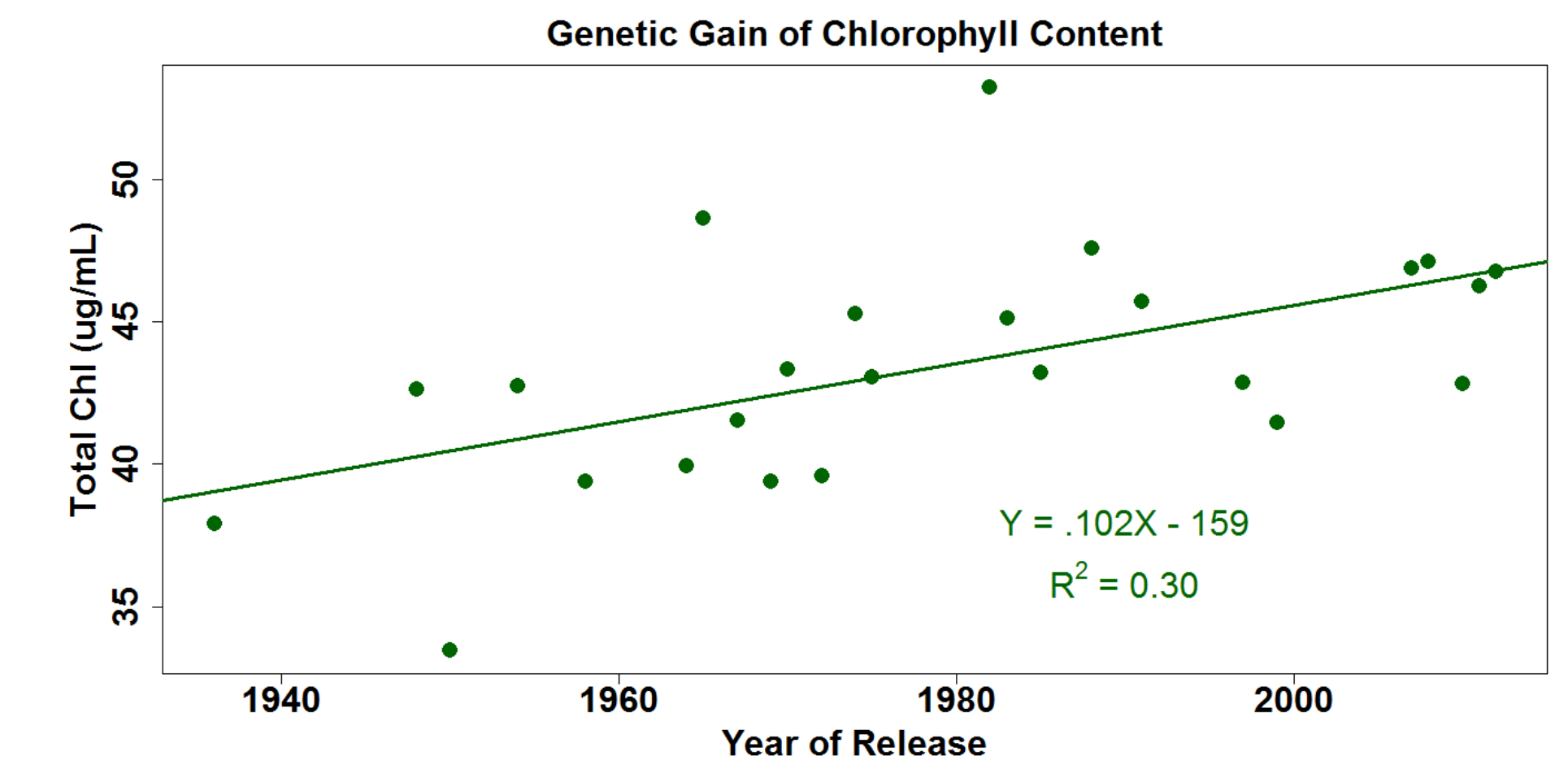


Figure 4. Linear regression of chlorophyll content over year of hybrid release (July 28, 2014).

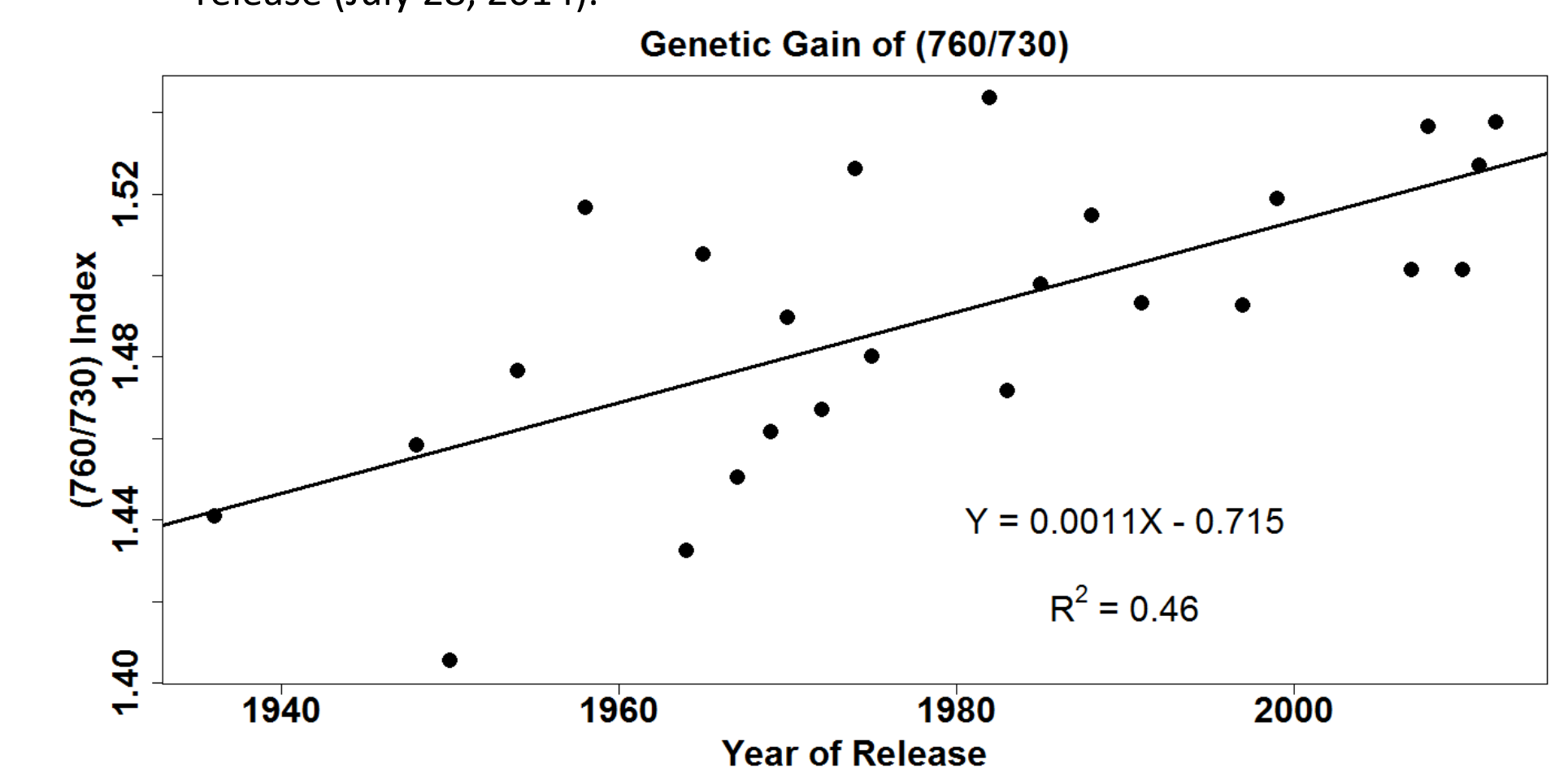


Figure 5. Linear regression of the (760/730) index over year of hybrid release (July 28, 2014).

Genetic Gain of Indices

At basically every sampling date across the growing season, the (760/730) index discovered significant genetic gains. Although most dates were significant, the best fits (determined by steep slopes and high coefficients of determination) peaked around flowering.

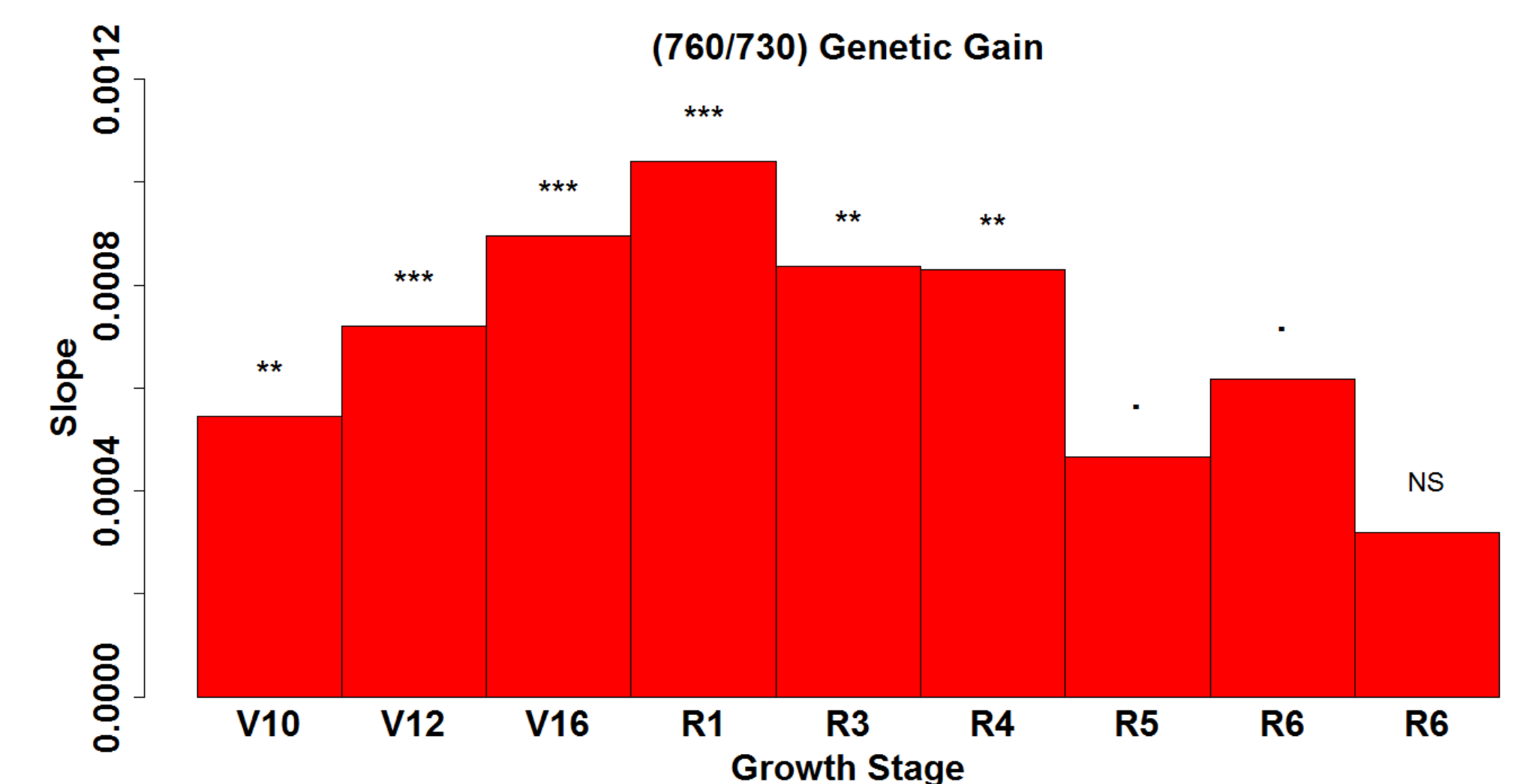


Figure 6. Slopes from the linear regression model of the (760/730) index over year of hybrid release for all measurement dates.

Conclusions

- Hyperspectral reflectance indices correlate well with leaf traits, primarily serving as a proxy for chlorophyll content and, in turn, gross primary production.
- Accompanying other agronomic traits, modern hybrids exhibit increased grain yield and chlorophyll content with decreased lodging and ASI.
- Throughout the growing season, the spectrometer could easily distinguish old and new hybrids.
- While most measurement dates showed significant genetic gains in index values, the maximum gains around flowering may represent the importance of that growth stage for enhanced production.

This research was supported by the UNL Life Sciences Competition and Nebraska Corn Board

Duvick, D. N., Smith, J. S. C., & Cooper, M. (2004). Long-term Selection in a Commercial Hybrid Maize Breeding Program. *Plant Breeding Reviews*, 24(2), 109-151.

Gitelson, A. A., Vina, A., Ciganda, V., Rundquist, D. C., & Arkebauer, T. J. (2005). Remote estimation of canopy chlorophyll content in crops. *Geophysical Research Letters*, 32(8).

Peng, Y., Gitelson, A. A., Keydan, G., Rundquist, D. C., & Moses, W. (2011). Remote estimation of gross primary production in maize and support for a new paradigm based on total crop chlorophyll content. *Remote Sensing of Environment*, 115(4), 978-989.

Winterhalter, L., Mistele, B., Jampatong, S., & Schmidhalter, U. (2011). High throughput phenotyping of canopy water mass and canopy temperature in well-watered and drought stressed tropical maize hybrids in the vegetative stage. *European Journal of Agronomy*, 35(1), 22-32.