THE EFFECT OF SHOOT MATURATION ON TOTAL BIOMASS PRODUCTION IN MAIZE

BY

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THESIS

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ABSTRACT

Corn (*Zea mays)* is a photosynthesis-efficient C4 grass, a group considered to be a potentially valuable source of lignocellulosic biomass. Previous studies have indicated the potential for higher total biomass production from maize genotypes with delayed shoot maturation, which accumulate more stover at the expense of grain yield. However, genetic variation for total biomass yields or their response to nitrogen has not been characterized extensively among maize hybrids with delayed shoot maturation. The objective of this study was to directly compare total biomass yields among a diverse group of maize hybrids where shoot maturation was delayed in two ways: either by crossing genetically diverse temperate-adapted inbreds with photoperiod-sensitive inbreds of largely tropical origin, or by overexpression of the maize *Glossy15* gene in different temperate-adapted hybrids. Biomass and nitrogen accumulation in stover and grain were evaluated for these genotypes in field trials on nitrogen-limiting (no supplemental fertilizer) or nitrogen-sufficient soils (200 kg N fertilizer per hectare). The results obtained demonstrate that use of either photoperiod-sensitivity genes or *Glossy15* can variably delay shoot maturation and reduce harvest index in a wide diversity of temperate-adapted backgrounds. When appropriately optimized for local adaptation, maize hybrids with delayed shoot maturation can produce higher total biomass yields under N-limiting conditions.

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CHAPTER 1

Genetic Variation of Nitrogen Utilization in Photoperiod-Sensitive Hybrids with Delayed Shoot Maturation

Introduction

Recent debates on climate change and global energy consumption has spurred considerable interest in the development of alternative and renewable energy sources. Among these sources, bioenergy is currently the only available technology capable of producing a replacement for transportation fuel, mostly in the form of ethanol. In the United States, approximately 12.1 billion gallons of corn grain ethanol was produced in 2009 (Service, 2010), which made up the majority of total ethanol produced nationwide. Rising concerns for the production food and fuel on agricultural lands and the energy costs associated with producing grain, however, favor the production of 'second generation' advanced biofuels from sources other than food crops. Possibilities that have been suggested include algae (Beer, 2009), organic wastes (Lastella, 2001) and lignocellulosic portions of crops, perennial grasses and forestry products (Perlack, 2005).

Maize (*Zea mays)* currently is the most commonly cultivated crop in the United States, with annual planting area of 80 million acres. The 2009 season yielded approximately 13,000 million bushels of grain, of which 3,200 million bushels were used to generate ethanol. The total amount of potential stover biomass produced from the same maize crop is estimated to be similar to grain yields, which could generate 50 gigaliters of ethanol if one-half of the possible stover was harvested for biofuels (Somerville, 2010). A previous study by Perlack *et.al* (2005) indicated that it should be

feasible to convert 30% of the U.S. liquid transportation fuels to biofuels by 2030. This same report projected that corn stover is likely to provide a significant supply of biomass for the second and third generation biofuels, due to its availability and ease of production. The wasted corn and corn stover already produced from a single year, with ground cover kept at 60%, could potentially account for 73 gigaliters of ethanol on a global scale, or approximately 4.7% of the world's gasoline consumption. (Kim, 2004)

The potential of maize as a lignocellulosic feedstock is also enhanced by the range of genetic variation available to breeders. King *et al* (1972) has demonstrated that maize originally grown in the tropics exhibits several physiological differences relative to temperate-adapted maize when grown in a temperate environment, such as delayed flowering time, increased stalk volume and plant size, and greater asynchrony between tasseling and silking. Each of these traits can be attributed to the sensitivity to photoperiod associated with longer daylength in the United States, and may contribute to higher total biomass yields. However, the direct use of tropical-adapted germplasm in temperate environments is limited by extreme delays in shoot maturation and senescence important for reducing moisture content of harvested biomass, as well as inferior agronomic traits such as greater susceptibility to diseases and insects. One potential solution to these issues to grow hybrids that combine the improved agronomics of temperate-adapted germplasm with the photoperiod sensitivity of tropical germplasm. A recent evaluation of such temperate-tropical maize hybrids indicates that they can potentially produce more total biomass than temperate-adapted maize grain hybrids, with the added benefits of lower nitrogen requirements and the accumulation of stalk sugars that can be more readily converted to biofuels (White et al., 2009).

Nitrogen is often the most limiting factor in the growth of maize and grain production. It is a critical component in protein synthesis as well as the development of photosynthetic apparati in plant leaves, affecting radiation interception and the growth of the plant as a whole. (Oaks, 1994) Deficiency in nitrogen can cause reductions in plant size, leaf area, grain yield, as well as accelerated senescence and faster drying of grain. Decreases observed in biomass production can often be attributed to N deficiency, as a result of loss in the amount of light intercepted, or the efficiency in which light energy is converted into biomass (Muchow, 1988). Applications of supplemental N fertilizer are widely used to increase grain yields of maize, but not all of the applied N is used by the crop, leading to significant economic costs for grain production and environmental concerns. The relatively high amounts of N fertilizer required to maximize maize grain yields limits further uses of corn as a sustainable biomass feedstock.

Nitrogen use in corn is most commonly determined by the Nitrogen Use Efficiency (NUE), traditionally defined as the amount of grain produced per unit of N supplied (Moll, 1982). Considerable variation in NUE has been observed among different genotypes, with a significant level of genotype by environment interaction (Presterl et al., 2003). NUE at high levels of nitrogen input is depends primarily upon the amount of nitrogen uptake, whereas at lower input levels the partitioning of N is considered more important (Moose and Below, 2008). NUE can be further described by two primary components: Nitrogen Uptake Efficiency (NUpE), which represents the amount of supplemental nitrogen taken up by the plant, and Nitrogen Utilization Efficiency (NUtE), defined as the ability of the plant to remobilize available N to reproductive sinks, respectively. In practice, NUpE is calculated as the ratio of the total N

in the plant (including grain and stover) at maturity and the nitrogen rate, while NUtE is calculated as the ratio between grain produced and the rate of nitrogen supplied (Moll, 1982). Physiological response to additional nitrogen can be divided into two broad types: N responsive and unresponsive. N-responsive genotypes show higher yields than unresponsive genotypes under high N levels, although the unresponsive genotypes do typically perform well under low N (Smiciklas and Below, 1990).

Modern maize hybrids have mostly been developed for high grain production under high soil N (Duvick, 1984). While a correlation has been documented between biomass accumulation and yield (Pedersen, 1996) and nitrogen deficiency has been shown to affect total biomass as well as grain, there is little information regarding the nitrogen response of maize in terms of vegetative biomass. In this study, we attempt to characterize genetic variation for nitrogen utilization in terms of total biomass and stover biomass, as well as the partitioning of N between grain and stover. The impact of photoperiod sensitivity on accumulation and partitioning of biomass and N is evaluated by comparing maize hybrids derived from crosses of temperate-adapted parents, tropical-adapted parents, and crosses between tropical and temperate-adapted parents. The results are considered in the context of the potential for hybrids from temperate by tropical parents as a feedstock for production of biofuels from lignocellulosic biomass.

Materials and Methods

Genetic Materials

Table 1 lists the 51 inbred lines tested as parents in this study, selected to represent a broad range of maize genetic diversity and adaptation. The inbred lines can

be divided into three major groups: historical photoperiod-insensitive (HPI), elite photoperiod-insensitive (EPI), and photoperiod-sensitive (PS). The HPI group includes the 14 photoperiod-insensitive and temperate-adapted founders of the maize Nested Association Mapping panel (NAM; Liu et al., 2003), and Illinois Low Protein 1 (ILP1), which exhibits high N utilization efficiency for dry matter production (Uribelarrea et al., 2007). The EPI group includes a collection of inbred lines that broadly represent genetic diversity and major heterotic groups in current elite temperate-adapted U.S. germplasm, as defined by Mikel and Dudley (2006). The PS group includes the 12 inbred founders of the NAM that exhibit delayed shoot maturation and are of primarily tropical origin. An additional five tropical lines were added on the basis of exhibiting poor grain yields (and hence possibly strong photoperiod sensitivity) in a survey of hybrids derived from temperate and tropical-derived hybrids when grown in North Carolina (Nelson et al., 2008). Each of the inbreds in the PS group flowered later than 90 days after planting in central Illinois, whereas all of the EPI and HPI inbreds flowered before 80 days after planting.

Six groups of hybrids were generated from crosses among the inbred lines in Table 1. Table 2 describes these hybrid groups and how many hybrids within each group were evaluated in the two years of the study. The majority of hybrids included the B73 inbred as female parent, which represents the "Stiff-Stalk" heterotic group that is the most commonly used as one of the parents in current commercial US hybrids. B73 was crossed to inbreds from the HPI and PS groups to assess genetic variation in biomass potential and N utilization among historical maize germplasm. Although B73 exhibits good general combining ability for grain yield, it is not currently considered an elite

inbred parent and may not produce the best hybrid for certain PS lines, thus another set of hybrids was generated between crosses of EPI and PS parents. The EPI parents used in these crosses were selected to span the range of maturity present in the collection, with FR1064 being the earliest (~65 days after sowing) and HBA1 being the latest (~80 days after sowing). Three hybrids between the ILP1 inbred and different PS parents were also evaluated. The final group of hybrids was generated from crosses between PS parents, which flowered between 95 and 110 days after sowing.

One goal of this study was to identify hybrids with superior total biomass yields. Selection for hybrids tested in 2009 was based on competitive total biomass yields obtained from a preliminary study conducted in 2008, relative to the high biomass hybrid B73 x Mo18W. After comparing data between genotypic groups from 2009, the EPI x PS group showed promise as sources of hybrids with higher total biomass, and more from this group were tested in 2010.

Experimental Design

Field experiments were conducted at the University of Illinois at Urbana-Champaign, IL, during the growing seasons of 2009 and 2010. Seeds were treated with fungicide and planted in 5.3 x 0.762 m plots with hand planters on June 15th 2009 and May 5th 2010, respectively. Planting was delayed in 2009 due to heavy rainfall. In 2009, the plants were grown at 20 plants per plot (approximately 28,000 plants per acre or 69,000 plants per hectare) in two replications. Plant density in 2010 was increased to 30 plants per plot (approximately 40,000 plants per acre, 100,000 plants per hectare) based on results from a pilot study conducted in 2009 with the B73 x Mo18W hybrid that

indicated optimal total biomass yields at the higher plant density. In both years, the experiments were grown in paired split-plots where each genotype was grown either without supplemental N fertilizer, or with 200 kgN/ha in the form of granular ammonium sulfate ($(NH_4)_2SO_4$) spread by hand evenly within the middle of the inter-row space. Weed control was performed by hoeing at approximately 30 days in both years. While no additional water was given in 2009, dry weather during the seed fill period in 2010 necessitated irrigation when signs of water stress were shown, e.g. lower leaves drying.

Four representative plants from the middle of each row were sampled in 2009, with the numbers reducing to 3 and 2 plants in plots with low stand count. Five plants were collected from each plot in 2010, to better estimate total biomass yields. The stover was separated from the ear, then weighed for fresh weight and shredded into chips using a mechanical shredder. Cloth bags were used to collect aliquots of the shredded stover, which were then weighed for the aliquot fresh weight and then placed to dry in hot-air ovens at 75^{o}C for 7 days. The aliquot is then re-weighed for aliquot dry weight and grounded to a fine powder (passing through a <2 mm mesh screen), a subsample of which was then collected in envelopes for further nitrogen concentration analysis using a Fissions NA 200 N Analyzer. Collected ears were dried at 35 ^{o}C in a forced-air oven to 10% moisture, then shelled using a mechanical sheller. Grain weight and cob weight were measured separately and added to the total plant weight. Grain nitrogen concentration was measured using Near Infrared Transmission spectroscopy (NIT) in 2009. Due to difficulties in acquiring an adequate amount of grain for the NIT process for many of the samples in 2009, however, the grain nitrogen analysis was changed to Near Infrared Reflectance Spectroscopy (NIR) in 2010. Values between the two methods are

comparable. Calculations used to estimate moisture adjustment values, total stover dry weight and nitrogen use efficiency are listed below.

$$Moisture\ adj = \frac{stover\ fresh\ weight}{aliquot\ fresh\ weight}\ x\ (aliquot\ fresh\ weight - aliquot\ dry\ weight)$$

$$Total\ stover\ DW = Total\ stover\ fresh\ weight - moisture\ adjustment\ value$$

$$Total\ N\ content = \frac{\%\ grain\ N\ x\ grain\ weight}{100} + \frac{\%\ stover\ N\ x\ stover\ dry\ weight}{100}$$

$$Total\ biomass = Total\ stover\ dry\ weight + total\ grain\ weight$$

$$Nitrogen\ utilization = \frac{Total\ biomass}{(Total\ nitrogen\ content)}$$

The PROC MIXED procedure in SAS was used to analyze the variation in total biomass, grain weight, stover dry weight, cob weight, year effect and nitrogen utilization, which were used to generate the ANOVA presented in Table 1.3, and to generate the comparison of least square means approximating the Tukey-Kramer t-test. The PROC CORR procedure was used to calculate Pearson correlation coefficients between variables in Table 1.4.

Results and Discussion

Environmental Effects on Biomass Production

Table 1.3 summarizes the impact of the major variables of growing season, N level, and genotype on estimates for measured biomass and N use components. Across all genotype groups and traits, differences among the 2009 and 2010 growing seasons were significant ($P = 0.0002$), except for nitrogen uptake and stover biomass among the hybrids with a PS parent. Table 1.4 shows that total biomass yields within each genotypic group were comparable in the two years of the experiment, despite differences in specific hybrids, plant density and climatic conditions. The 2009 growing season was characterized by continuous above-average precipitation, resulting in delayed planting, poor stands in some areas of the field, lodging and a delayed harvest date. The 2010 season, on the other hand, was relatively dry, which accelerated post-flowering senescence and led to a higher incidence of stalk breakage and dropped leaves at harvest. These losses likely offset the potentially higher yields that might be expected with increased plant density. Overall, the 2010 season and planting density would be considered most relevant to a typical maize production season in the U.S. Corn Belt.

Supplemental N increased all biomass measures and particularly harvest index in the B73 x HPI and B73 x EPI hybrids (Tables 1.3 and 1.4), which is expected considering past selection for grain yields that are highly responsive to N fertilizer. However, no effect of N supply on stover production was detected in the hybrids with PS parents, some of which did show dramatic increases in grain yield with N. N fertility had no effect on grain weight in the PS x PS hybrids, probably because these hybrids produced very

little grain. Thus, reductions in harvest index meditated by delayed shoot maturation appear to offer greater environmental stability to total biomass yields.

As expected, N fertilizer significantly increased plant N accumulation for each genotypic group in both years. There was a trend for higher N accumulation in 2010 compared to 2009 for most genotypic group, particularly in the unfertilized plots, which may be attributed to either the higher plant density or greater soil N availability to plants during the 2010 growing season. Table 1.5 shows that total nitrogen content per plant is strongly correlated to total biomass in photoperiod insensitive groups, whereas the same relationship is less significant for hybrids with a PS parent, and was non-significant for PS x PS hybrids. Thus, as harvest index declines, the nitrogen requirement to maximize stover biomass compared to grain yields likely also decreases, such that high levels of N fertilization may offer limited benefit.

Genetic Variation in Biomass Accumulation

Results from the 2009 experiment suggested that the average total biomass across genotype groups is not significantly different under high additional nitrogen input (Table 1.4). However, under no additional nitrogen input, the PS hybrids exhibited higher average total biomass compared to the PI hybrids, particularly the PS x PS hybrids. The standard deviations for measured traits in 2009 indicate that similar amounts of phenotypic diversity were present within each genotypic group, except for the HPI x PS group that contained only four hybrids sharing the same ILP1 tester. Although a similar number of hybrids from most genotype groups were planted again in 2010 under higher density, the specific genotypes included varied due to difficulties in seed production. In

addition, the EPI x PS group was enlarged to better document the influence of delayed shoot maturation in genetic backgrounds that have been strongly selected for grain yield, which is reflected in the higher standard deviations for measured traits in the EPI x PS group in 2010 compared to 2009.

Considering the entire experiment (Table 1.3), the variation due to hybrid was typically not significant for the genotypic groups where B73 was crossed as female to diverse parents. It is known that B73 exhibits strong general combining ability for grain yield, and this study indicates this is also true for total biomass even when shoot maturation is delayed. The effect of male parent was significant for total biomass in the B73 x EPI group, which likely reflects differences in specific combining ability with EPI parents that represent a number of heterotic patterns, including crosses within the Stiff-Stalk group that contains B73. However, the relatively small impact of PS parent on observed variation for biomass traits suggests that delayed shoot maturation was more important than other types of genetic variation.

Although the effect of hybrid on total biomass was not detected as significant for the EPI x PS group in the experiment overall (Table 1.3), hybrid was significant for grain, stover, and cob yields. Importantly, the effect of the PS male parent was significant for all biomass measures, but not the EPI female parents. These findings have practical implications for choosing parents to further improve hybrids derived from crosses between photoperiod sensitive and insensitive genotypes. It appears that the source and degree of delayed shoot maturation is more critical than temperate genetic background for optimizing the best total biomass yield, and that EPI parents spanning a wide range of relative maturities may be equally useful producing hybrids with higher total biomass

potential. Thus breeders appear to have many options for choice of EPI parent, and could choose those with higher yields in seed production. We also observed a strong effect of both parents on total biomass for the PS x PS group, which likely reflects their broad diversity, possible differences in modes of delayed shoot maturation, and the importance of defining specific combining ability for PS x PS hybrids in temperate environments.

Figures 1.1 and 1.2 better illustrate the continuous genetic variation for total biomass among 88 individual hybrids and genotypic groups from the 2010 experiment. Similar trends were observed in 2009. In the absence of supplemental N, each genotypic group contained hybrids with total biomass yields ranging from 10 to 20 Mg/ha (Figure 1.1), but only hybrids with PS parents produced yields greater than 20 Mg/ha. The one exception was the B73 x NC350 hybrid, which although classified in the HPI group, NC350 is one of the latest-maturing HPI inbreds. As indicated by their higher average biomass yields (Table 1.4), the PS x PS group had the greatest number (six) of high biomass hybrids. In addition, three B73 x PS and five EPI x PS hybrids also produced greater than 20 Mg/ha biomass without supplemental N. Interestingly, the Mo18W parent that had been identified in prior preliminary studies of B73 x PS hybrids as having highest biomass at low N was represented in each of the B73 x PS, EPI x PS, and PS x groups in 2010. Other promising PS parents include CML139, CML52, and Ki11. The FR1064: cmsT x Mo18W hybrid is also noteworthy because of the cytoplasmic male sterility of its female parent, which may offer advantages in further reducing grain production as a hybrid due to the lack of pollen production.

Figure 1.2 shows the estimated yields for the same 88 hybrids grown with 200 kg N per hectare. The overall pattern of continuous variation within and relative ranking

among genotypic groups is similar to that observed at low N and the means reported in Table 1.4, but total biomass yields are shifted upwards to range from 15 to nearly 50 Mg/ha. Using 25 Mg/ha as a threshold, one HPI x PS, two B73 x HPI, two B73 x PS, five PS x PS, and 12 EPI x PS hybrids can be considered to have the highest potential biomass yields. Importantly, five of the highest-yielding hybrids at low N (Mo18W x CML139, CML52 x Tzi3, PHG84 x CML52, B73 x CML139, and B73 x NC350) are also found to produce very high yields with supplemental N. We consider the estimates for the three hybrids yielding greater than 35 Mg/ha to likely be inflated, possibly due to poor stands and thus less interplant competition.

The observed total biomass yields are competitive with those that might be obtained by growing leading commercial grain hybrids in the U.S. Corn Belt. Based on an observed average yield of 210 bushels per acre at 15% grain moisture from 115 hybrids evaluated in 2010 at the nearby Urbana location (http://vt.cropsci.illinois.edu/), an average total biomass yield of approximately 22.5 Mg/ha would be expected using a value of 0.5 for harvest index. The mean total biomass yields for each of the PS hybrid groups we evaluated in 2010 met or exceeded this value (Table 1.4).

The Nitrogen Requirement for Total Biomass Production

One of the most important issues in bioenergy crop production is the amount of resources needed to produce the crop versus the possible energy output from the harvest. In maize, nitrogen is one of the most limiting factors of growth, and arguably one that causes the most amount of concern, accounting for over 30% of the total energy expended in production (Kim, 2009). Previous studies with maize grain hybrids (Lavigne,

2007) have shown that a significant amount of energy must be expended in order to produce higher levels of biomass, which includes both grain and stover. Investigations into the amount of stover available in the U.S. have also assumed an equal partitioning of total biomass between stover and grain (Graham, 2007) or that the amounts of nitrogen fertilizer required to optimize corn stover are similar to those applied in grain production (Somerville, 2010). Our experiments suggest that these assumptions may not hold when applied to hybrids with much lower harvest index.

We calculated the amount of plant nitrogen uptake (Table 1.5), which on a per area basis serves as an estimate for potential N removal from harvested biomass. For the plots with no supplemental N, average plant N uptake is an indicator of how much N could be supplied by the soil. The field site used in our experiments has been managed for six years and three corn-soybean cropping cycles for low N fertility, and was estimated to provide between 75 and 150 kg N/ha across years and different genotypic groups (Table 1.4). The mean N removal across all low N plots in the experiment was approximately 100 kg N/ha. These soil N levels are already sufficient to produce relatively high amounts of total biomass, at least 10 Mg/ha for the worst hybrids grown in 2010. The values for N removal when plants are grown without supplemental fertilizer also suggests the amount of supplemental N required to sustain similar biomass yields from this production environment. This "N replacement" value of 100 kg N/ha is similar to current recommended rates of N fertilizer application for corn production in central Illinois (150-200kg N/ha), when efficiencies of N fertilizer use from 50-67% are considered. The uptake efficiency of fertilizer N can be estimated by subtracting the amount of N removed from plants grown at low N from that removed following N

application, then dividing by the amount of applied N fertilizer. When averaged across all 280 paired-treatment plots in the two years of these trials, N uptake efficiency was estimated at 62.5%. Based on the more relevant 2010 experiment, mean N uptake efficiencies were 41% for 21 PI hybrids, but increased to 69% for the 67 PS hybrids. The higher N uptake efficiency of PS hybrids improves the energy balance for lignocellulosic biofuel production from maize and may alleviate some of the concerns regarding soil N removal and the sustainability of biofuel production from corn stover.

Variation in Nitrogen Utilization

N utilization is the other major component of overall N use efficiency. We calculated nitrogen utilization for total biomass rather than only grain yield as typically reported, to more directly compare productivity among genotypes that are expected to differ widely in biomass partitioning between stover and grain. The observed variation for nitrogen utilization is greater at 0 kgN/ha than 200 kgN/ha (Figure 1.3, $p < 0.01$). While NUE and N utilization are negatively correlated for both the 0 kgN/ha and 200 kgN/ha treatments (-0.57058 and -0.72759 at alpha = 0.05, respectively), the relationship between NUE and N Utilization is stronger at a high level of additional nitrogen input (Figure 1.4). This suggests that selection for high grain yields is highly correlated with increases in total biomass when grown in locations with high levels of nitrogen, but may not necessarily be applicable for total biomass in low-input conditions.

Total biomass is highly correlated with grain yield (Table 1.5), particularly in PI hybrids, which may explain the significant negative correlation between nitrogen utilization and total biomass in these groups. N utilization is not significantly correlated

with total biomass in the PS hybrid groups. N utilization values show significant variation between the two years in the B73 hybrid groups (Table 1.3). However, hybrids in the same genotypic group tend to show the same level of N utilization as the effect of hybrid on variation for N utilization was not significant (Table 1.3).

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Tables

Table 1.1 List of inbred parents by group.

Genotype Groups		
Photoperiod Sensitive (PS)	Elite Photoperiod-Insensitive (EPI)	Historical Photoperiod-Insensitive (HPI)
CML52	B73	ILP1
CML69	PH207	B97
CML139	FR1064	CML103
CML228	HBA1	CML322
CML246	Ki11	Hp301
CML247	LH1	Il14H
CML277	LH123	Ky21
CML333	LH123Ht	M37W
CML349	LH156	Mo17
GP 273	LH60	MS71
Ki3	LH82	NC350
Ki11	PHG35	NC358
Mo18W	PHG39	Oh43
Tx303	PHG47	Oh7B
Tzi3	PHG84	P39
Tzi8	PHJ40	
	PHT55	
	PHW65	
	PHZ51	
	PVP1	

Table 1.2 Number of hybrids from each genotype group evaluated in field trials during 2009 and 2010.

Genotype Group	2009	2010	2009 and 2010
B73 x EPI	11	8	7
B73 x HPI	13	13	9
B73 x PS	15	9	8
EPI x PS	7	48	4
HPI x PS	4	3	1
PS x PS	2	7	0

Table 1.3 Sources of variation for physiological traits measured in 2009 and 2010.

Traits	Year	Nitrogen	Hybrid	Female Parent	Male Parent
B73 x HPI					
Total biomass	**	**	NS	-	NS
Stover dry weight	**	**	**	-	**
Grain weight	*	**	NS	-	NS
Cob weight	**	**	NS	-	NS
Nitrogen Uptake	NS	**	NS	-	NS
Nitrogen Utilization	**	**	NS	-	NS
B73 x EPI				-	
Total biomass	**	**	NS	-	**
Stover dry weight	**	**	NS	-	NS
Grain weight	**	**	NS	-	NS
Cob weight	**	**	NS	-	*
Nitrogen Uptake	NS	**	NS	-	NS
Nitrogen Utilization	**	**	NS	-	NS
B73 x PS				-	
Total biomass	**	**	NS	-	NS
Stover dry weight	NS	NS	NS	-	NS
Grain weight	**	**	NS	-	NS
Cob weight	**	**	NS	-	NS
Nitrogen Uptake	NS	**	NS	-	**
Nitrogen Utilization	**	**	NS	-	NS
PS x PS					
Total biomass	**	**	**	**	**
Stover dry weight	NS	NS	**	**	**
Grain weight	**	NS	NS	NS	NS
Cob weight	**	**	**	**	NS
Nitrogen Uptake	NS	**	NS	NS	NS
Nitrogen Utilization	NS	**	NS	NS	NS
HPI x PS					
Total biomass	**	**	NS	NS	**
Stover dry weight	NS	NS	NS	*	**
Grain weight	**	**	NS	**	NS
Cob weight	*	**	*	NS	NS
Nitrogen Uptake	NS	**	NS	NS	NS
Nitrogen Utilization	NS	**	NS	NS	NS
EPI x PS					
Total biomass	*	**	NS	NS	**
Stover dry weight	NS	NS	**	NS	**
Grain weight	**	**	**	NS	**
Cob weight	**	**	**	*	**
Nitrogen Uptake	NS	**	NS	NS	NS
Nitrogen Utilization	NS	**	NS	NS	NS

* denotes significance at $\alpha < 0.1$

** denotes significance at $\alpha < 0.05$

NS denotes non-significant, $\alpha > 0.1$

Table 1.4 Means and standard deviations (SD) for total biomass yields, harvest index, and N removal from maize hybrids evaluated in 2009 and 2010.

No supplemental N fertilizer

	2009						2010					
	Total Biomass (Mg/ha)		Harvest Index		Total N Removal (kg/ha)		Total Biomass (Mg/ha)		Harvest Index		Total N Removal (kg/ha)	
Genotype Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
B73 x HPI	14.9	4.3	0.36	0.18	87	32	16.1	2.5	0.42	0.07	130	25
B73 x EPI	15	3.8	0.31	0.15	82	27	16.8	2.7	0.39	0.06	128	23
B73 x PS	16.5	4.3	0.31	0.13	74	23	20.1	6.6	0.14	0.13	149	79
EPI x PS	16.5	3.4	0.32	0.04	78	22	15.7	4.3	0.15	0.13	103	42
HPI x PS	18.6	0.5	0.35	0.04	86	4	14.8	4.5	0.09	0.11	75	15
PS x PS	22.7	4.3	0.13	0.1	113	66	21.7	7.3	0.02	0.05	129	56

200 kg supplemental N fertilizer per hectare

	2009						2010					
	Total Biomass (Mg/ha)		Harvest Index		Total N Removal (kg/ha)		Total Biomass (Mg/ha)		Harvest Index		Total N Removal (kg/ha)	
Genotype Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
B73 x HPI	21.3	3.4	0.5	0.04	231	54	20.4	3.4	0.49	0.03	209	32
B73 x EPI	21.0	4.1	0.45	0.05	228	54	21.6	4.1	0.47	0.04	224	51
B73 x PS	19.0	4.6	0.43	0.06	186	54	22.9	4.7	0.39	0.11	235	67
EPI x PS	21.4	2.4	0.38	0.08	235	31	22.7	7.5	0.35	0.14	250	93
HPI x PS	19.0	1.0	0.38	0.03	162	6	24	4	0.23	0.21	256	104
PS x PS	21.3	1.4	0.18	0.04	154	8	24.2	7.7	0.06	0.06	266	86

Table 1.5 Correlations between phenotypic traits, nitrogen content and nitrogen utilization measured in 2009 and 2010.

	Stover weight	Grain weight	Cob weight	Total Nitrogen Content	Nitrogen Utilization
B73 x HPI					
Total Biomass	0.85**	0.93**	0.85**	0.91**	-0.62**
Stover weight		0.63**	0.64**	0.71**	-0.27**
Grain weight			0.94**	0.92**	-0.77**
Cob weight				0.84**	-0.67**
B73 x EPI					
Total Biomass	0.84**	0.96**	0.92**	0.95**	-0.83**
Stover weight		0.67**	0.67**	0.83**	-0.53**
Grain weight			0.93**	0.91**	-0.90**
Cob weight				0.82**	-0.78**
B73 x PS					
Total Biomass	0.68**	0.89**	0.87**	NS	NS
Stover weight		0.28*	NS	NS	NS
Grain weight			0.85**	0.34**	NS
Cob weight				NS	NS
EPI x PS					
Total Biomass	0.73**	0.84**	0.81**	0.61**	-0.15
Stover weight		0.26**	0.38**	NS	0.186*
Grain weight			0.82*	0.78**	-0.36**
Cob weight				0.58**	-0.26**
HPI x PS					
Total Biomass	0.63**	0.72**	0.83**	NS	NS
Stover weight		NS	0.38	NS	NS
Grain weight			0.68**	NS	NS
Cob weight				NS	NS
PS x PS					
Total Biomass	0.92**	0.74**	0.84**	NS	NS
Stover weight		0.44*	0.60*	NS	NS
Grain weight			0.90**	0.51**	NS
Cob weight				0.48**	NS

* denotes significant difference at $\alpha < 0.1$
** denotes significant difference at $\alpha < 0.05$
NS denotes not significantly different, $\alpha > 0.1$

Figures

Figure 1.1 Distribution of total aboveground biomass for maize hybrids from six genotypic groups, grown without supplemental N in 2010. Specific hybrids producing the highest total biomass are named on the plot, color-coded by their genotypic group as indicated on the graph.

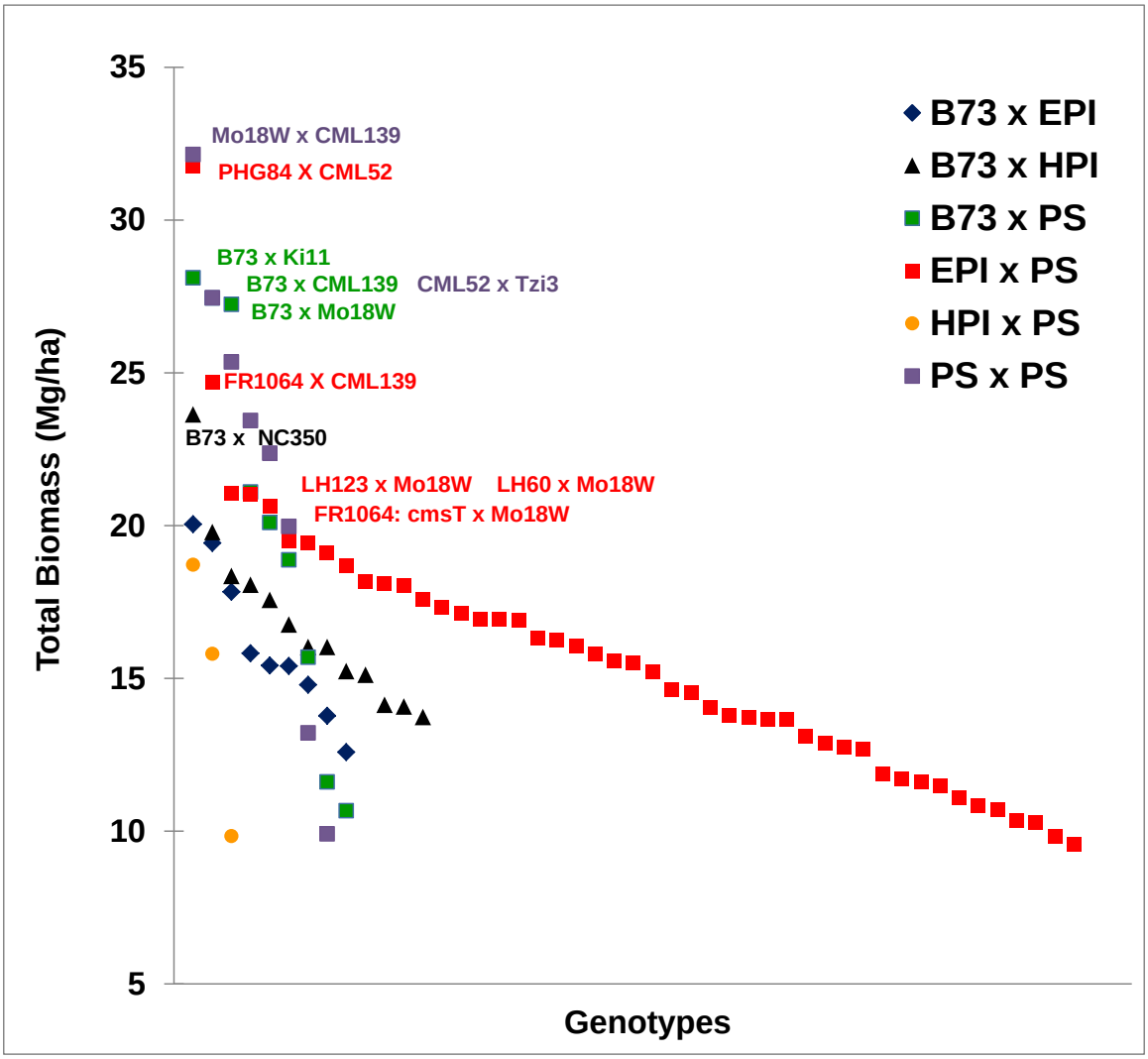


Figure 1.2 Distribution of total aboveground biomass for maize hybrids from six genotypic groups, grown with rate of 200 kg supplemental N per hectare in 2010. Specific hybrids producing the highest total biomass are named on the plot, color-coded by their genotypic group as indicated on the graph.

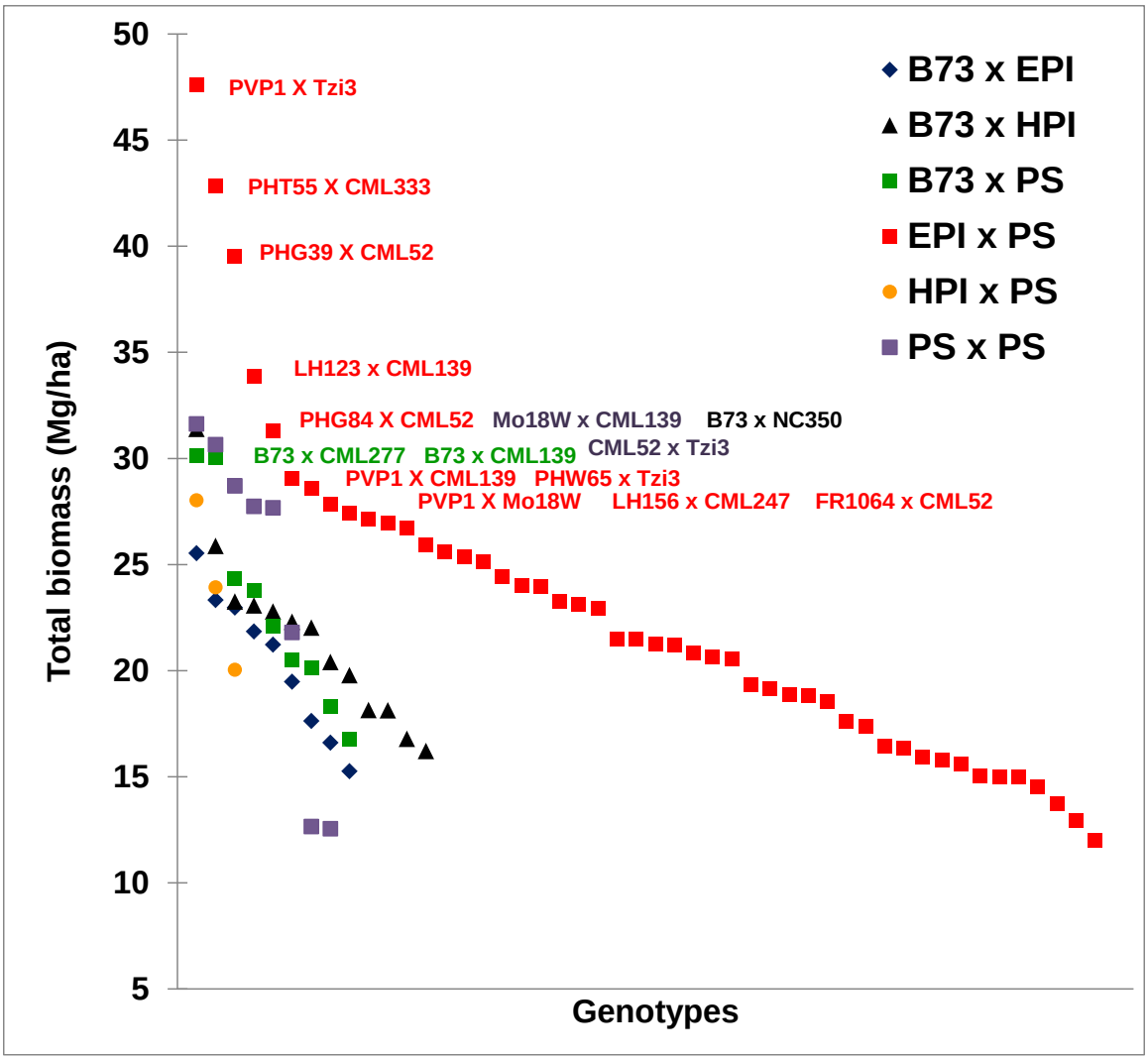


Figure 1.3 Comparison of nitrogen utilization for 52 diverse maize hybrids grown with either no supplemental N or 200 kg N/ha in 2009.

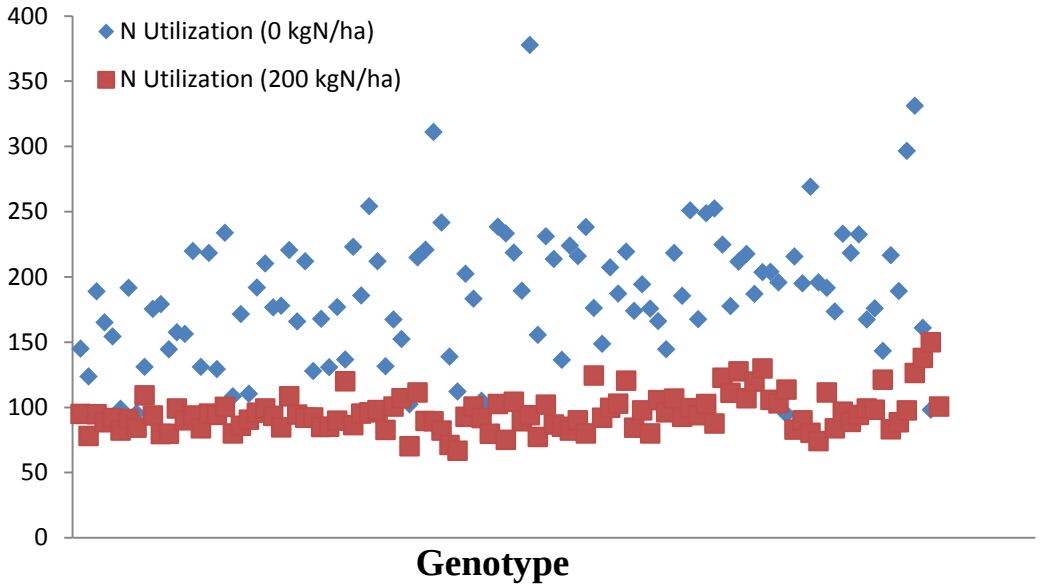
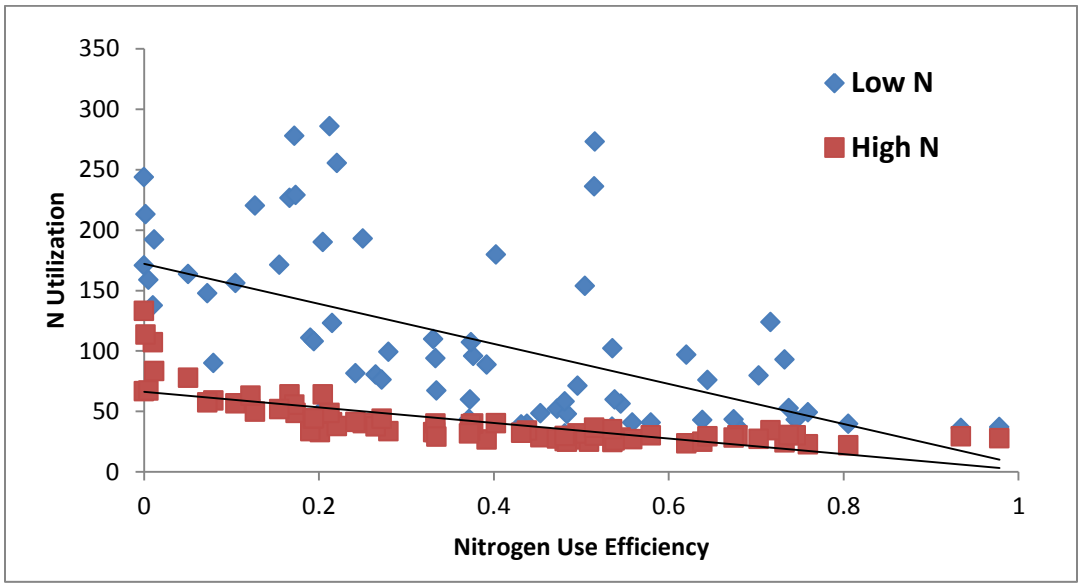


Figure 1.4 Scatter plot of relationship between Nitrogen Utilization and Nitrogen Use Efficiency for 52 diverse maize hybrids grown with either no supplemental N or 200 kg N/ha in 2009. Linear fit of relationships between N utilization and NUE is shown for both N levels.



CHAPTER 2

The Effect of *Glossy15* Overexpression on Biomass Production

Introduction

The regulation of flowering time is one of the strategies evolved to ensure or increase reproductive success in plants, allowing them to sense seasonal and environmental cues beneficial to the transition from vegetative to reproductive phase. The ability to sense and respond to changes in the relative length of day to night, referred to as photoperiodism, is a flowering time control mechanism used by many plant species, including corn. The transition from vegetative growth phase to reproductive phase may cause a shift in source-sink capacity between the production of photosynthetic organs and reproductive tissues, normally reducing the sink capacity of the leaves and increasing the capacity of the reproductive organs. Studies in tobacco (Salehi, 2005), *Xanthium canadense* (Shitaka, 1998) and *Brachyopodium distachyon* (Schwartz, 2010) show that alterated flowering time due to photoperiod control can lead to shifts in the growth of the vegetative and reproductive parts, i.e. early-flowering transgenes may lead to decreased vegetative growth, late-flowering transgenes may lead to increased vegetative biomass production while reducing the time period available for reproductive growth, resulting in altered or smaller reproductive organs. While this is undesirable in maize hybrids selected for grain production, the negative relationship between the two is a promising area of research for increasing total biomass production.

Maize *(Zea mays.)* is a short-day plant, i.e. the shorter the day length, the earlier the flowering date becomes. This is due to the tropical origin for the domestication of

teosinte, with modern temperate cultivars having been gradually adapted to the climate and day lengths of the temperate regions (Wang, 2010). Flowering is initiated in maize as the shoot apical meristem transitions from vegetative to reproductive development, and a few genes affecting this change have been identified. One of these genes, *Glossy15 (g115)*, is an *APETALA2*-like gene that is involved in the maintenance of juvenile leaf identity and delaying shoot maturation (Moose and Sisco, 1994; Moose and Sisco, 1996). Previous work with *AP2*-like genes that function in the reproductive phase change pathway have shown them to be post-transcriptionally regulated by *microRNA172* (*miR172*) in order to induce flowering, whereas *miR172* itself is regulated by photoperiod (Aukerman and Sakai, 2003; Schmid et al, 2003). Transgenic overexpression of *TARGET OF EAT1* (*TOE1)*, an *AP2*-like gene, has resulted in late-flowering *Arabidopsis* which behaves similarly to photoperiod-sensitive lines (Jung, 2007). Previous studies have shown that transgenic maize plants overexpressing *Glossy15* (*Gl15-TG)* exhibit delayed phase change, where more leaves with juvenile identity are produced (Lauter, 2005). Floral induction is also suppressed by *Glossy15* expression, which leads to later flowering and morphological changes in the tassels and ears that suggest greater meristem determinacy. Ears from *Gl15-TG* plants have reduced seed set, leading to lower grain production. However, with a longer time frame for vegetative growth, *Gl15-TG* plants produce greater stover biomass compared to non-transgenic isolines. When coupled with lowered grain yields, *Gl15-TG* plants possess similar features to photoperiod sensitive cultivars, raising the possibility that *Gl15-TG* plants may produce greater total biomass yields and exhibit different responses to nitrogen compared to their non-transgenic counterparts.

This study evaluated 17 *Gl15-TG* hybrids in order to document changes in total biomass, nitrogen use and biomass partitioning in relation to delayed shoot maturation. We also evaluated these hybrids for their potential as bioenergy feedstocks compared to photoperiod-sensitive hybrids derived from crosses between tropical- and temperate-adapted parents, or between tropical parents. Furthermore, by comparing inbreds and hybrids expressing *Gl15-TG* to their normal isolines that lack *Gl15-TG*, the effect of *Gl15-TG* and its delayed shoot maturation on the degree of heterosis for total biomass was examined.

Materials and Methods

Genetic Materials

A preliminary study was conducted in 2009 using six pairs of isogenic hybrids that either lacked or contained the *Gl15-TG* transgene. The hybrid backgrounds were B73 x Mo17, B73 x H99, FR1064 x H99, FR1064 x Mo18W and B73 x Mo18W. The latter two are promising photoperiod-sensitive hybrids (Chapter 1), which permitted analysis of delays in shoot maturation condition by *Gl15-TG* in combination with photoperiod-sensitivity.

Based on the results of 2009, another experiment was conducted in 2010 with 16 transgenic hybrids and 7 inbreds, with another 7 non-transgenic hybrids as controls. The transgenic hybrids used in the controlled experiment were B73 x H99: *Gl15-TG* and FR1064 x H99: *Gl15-TG*. Other hybrids consisted of FR1064: *Gl15-TG* crossed to 9 recent elite temperate-adapted inbreds and Mo18W x B73: *Gl15-TG* as comparison

benchmark. The inbreds (B73, H99, Mo17 and FR1064) used as parents in making *Gl15-TG* and non-transgenic control hybrids were also included.

Phenotypic Measurements

Field experiments were conducted at the research farms at the University of Illinois at Urbana-Champaign during the planting seasons of 2009-2010. The planting dates were on June 15th 2009 and May 5th 2010, respectively, due to heavy rainfall in the 2009 season leading to late planting. The seeds were pre-treated with fungicide before being hand-planted in 5.3m one-row plots with 75cm row spacing for 2009 and four-row plots of the same length and spacing in 2010. In both years, the hybrids in the experiments were subjected to two nitrogen treatments at 0 kgN/ha and 200 kgN/ha, respectively, while all inbreds were grown under no additional nitrogen input (0 kgN/ha). The additional nitrogen was supplied by hand in the form of ammonium sulfate ($(NH_4)_2SO_4$) in the inter-row space at 30 days. Weed control was performed by hand hoeing at approximately 30 days in both years and subsequently re-performed when necessary. The planting density in 2009 was 20 plants per plot (28,000 plants per acre, 69,000 plants per hectare), and increased to 30 plants per plot (40,000 plants per acre, 100,000 plants per hectare) in 2010 to more accurately reflect plant densities that are expected to be employed with maize grown for high total biomass.

Sampling was conducted as the plants reached full maturity (R6 stage) by collecting 4 plants from the middle of each row, which was increased to 5 plants in each row in 2010. The stover and ears were collected separately. The whole plot stover was measured for whole plot stover fresh weight before being mulched into chips using a

mechanical shredder. Aliquots of the shredded stover were stored in cloth bags, which were weighed for fresh aliquot weight and then oven-dried for 7 days at 75^{o}C. The dried aliquots were then reweighed for the dry aliquot weight, then ground to pass through a <2mm mesh screen using a Wiley laboratory mill. Subsamples of the ground stover were then collected in envelopes for nitrogen concentration analysis using a Fissions NA 200 N Analyzer. Whole plot ears were dried at 35^{o}C in a forced-air oven to 10% moisture, then mechanically shelled. Grain weight and cob weight were measured separately and added to stover to estimate total plant weight. The grain nitrogen concentration was measured using Near Infrared Transmission spectroscopy (NIT) in 2009. However, the transgenic hybrids produce significantly less seed than their non-transgenic counterparts, which may be insufficient for NIT analysis. Thus, in 2010 grain nitrogen concentrations were instead estimated using Near Infrared Spectroscopy in reflectance mode (NIR), after grain was ground to a fine powder with a mechanical mill.

Stover dry weight was obtained from the difference between the moisture adjustment value and stover fresh weight in the following equations.

$$Moisture\ adj = \frac{stover\ fresh\ weight}{aliquot\ fresh\ weight}\ x\ (aliquot\ fresh\ weight - aliquot\ dry\ weight)$$

$$Total\ stover\ DW = Total\ stover\ fresh\ weight - moisture\ adjustment\ value$$

Total N content and N of the plot was calculated with the following equation :

$$Total\ N\ content = \frac{\%\ grain\ N\ x\ grain\ weight}{100} + \frac{\%\ stover\ N\ x\ stover\ dry\ weight}{100}$$

Total biomass is calculated as the sum of stover dry weight (including cobs) and total grain weight. Nitrogen utilization is calculated as the ratio between total biomass and total nitrogen content.

The difference in total biomass, stover weight, grain weight, nitrogen content and nitrogen utilization between the non-transgenic and transgenic genotypes were analyzed using a one-tailed Student's T-test for two-point sample with equal variance, except in the case of the temperate *Gl15-TG* x Tropical group, where a pairwise comparison is used instead. We also estimated the percentage of heterosis for total biomass and stover dry weight between inbreds and hybrids with the following equation:

$$\%\ Heterosis = \frac{F1\ Value - Midparent\ Value}{Midparent\ Value}\ x\ 100$$

Results and Discussion

Glossy15 Effect on Total Biomass Production

Figures 2.1 and 2.2 show total biomass yields, partitioning between stover and grain, and plant N accumulation for *Gl15-TG* hybrids and their non-transgenic isolines grown in 2009. The effect of *Gl15-TG* was evaluated in six different hybrid backgrounds at two different levels of soil N. The FR1064 x H99, FR1064 X Mo17, B73 x H99 and B73 x Mo17 hybrids are representative of the Stiff-Stalk by Lancaster heterotic pattern used in the U.S. Corn Belt. These hybrids vary in relative maturity, with FR1064 x H99

being the earliest by about one week compared to the latest hybrid, B73 x Mo17. Two additional hybrid backgrounds were included where the same temperate-adapted female parents (FR1064 and B73) were crossed to the photoperiod-sensitive inbred Mo18W, which flowered 10 days later than B73 x Mo17.

As previously reported (Lauter et al., 2005), *Gl15-TG* delayed vegetative phase change and flowering time in each hybrid background, with *Gl15-TG* hybrids flowering approximately 10 days later than their non-transgenic isolines. As expected, *Gl15-TG* decreased harvest index in each hybrid background due to greater stover biomass production at the expense of grain. The decreases in harvest index were more pronounced in hybrids grown in low N compared to high N, due to the positive response of grain yield to N fertilizer. The delayed shoot maturation of *Gl15-TG* hybrids increased total biomass of the FR1064 x H99, FR1064 x Mo17, and B73 x H99 hybrids grown at low N, but had minor effects on biomass yields in the other hybrid backgrounds. In the presence of supplemental N, one dose of *Gl15-TG* increased total biomass for the B73 x H99 and B73 x Mo17 hybrid backgrounds, but either had no effect (FR1064 x H99) or conditioned slight decreases in total biomass. Within the B73 x Mo17 background, *Gl15-TG* homozygotes show a greater decrease in harvest index compared to hybrids hemizygous for *Gl15-TG*. The homozygous *Gl15-TG* hybrid produced slightly lower total biomass yields relative to the *Gl15-TG* hemizygous hybrid, but are still higher than the non-transgenic control. This observation suggests that some delay in shoot maturation programmed by *Gl15-TG* can be beneficial to total biomass yields, but that longer delays due to further increases in *Gl15-TG* activity may be detrimental.

Within the two related hybrids sharing Mo18W as a photoperiod-sensitive parent, the presence of *Gl15-TG* further depressed harvest index and also resulted in slight decreases in total biomass yields. This effect was observed under both levels of N fertility. These results suggest that the delay in shoot maturation resulting from *Gl15-TG* acts in a largely additive manner with the genes for photoperiod-sensitivity (currently unknown) that are present in Mo18W. The decreases in total biomass yields observed in when *Gl15-TG* is combined with photoperiod sensitivity, relative to either *Gl15-TG* or photoperiod sensitivity alone, are consistent with the interpretation that delayed shoot maturation can increase total biomass yields up to some threshold, beyond which further delays can essentially eliminate grain production and result in lower total biomass.

Figures 2.3 and 2.4 show the results obtained from a similar study conducted in 2010, the main differences being higher plant density compared to 2009 and the inclusion of additional *Gl15-TG* hybrids where the three recent elite inbreds that flower later than B73 were chosen as testers. For comparison, biomass yields and partitioning are also shown for three hybrids derived from crosses of photoperiod-sensitive parents that exhibit high biomass potential. Interestingly, some of the trends observed for the 2009 experiment were reversed in 2010. Most notable that for the FR1064 x Mo18W background, total biomass production was greater for the *Gl15-TG* hybrid without N fertilizer, and for the *Gl15-TG* hybrid provide N fertilizer. Comparison of the *Gl15-TG* hybrids with high-yielding photoperiod-sensitive hybrids shows that without additional nitrogen, the *Gl15-TG* hybrids produced less total biomass than the photoperiod-insensitive hybrids, but also made more grain. Grain yield in the transgenic group remains low compared to the non-transgenic controls, although it is increased in the 200

kgN/ha treatment. Stover yield in *Gl15-TG* transgenic plants increased when compared to the non-transgenic in most of the genotypes tested, with the exception of FR1064 x H99 and FR1064 x CML333. The group of three recent elite parents of grain hybrids showed fairly low biomass and little grain production when testcrossed to FR1064: *Gl15-TG*, even when provided 200 kgN/ha.

Figures 2.1 through 2.4 also present plant N accumulation for each of the hybrids evaluated. As expected, N fertilizer led to increases in plant N accumulation. Changes in plant N accumulation in response to *Gl15-TG* or photoperiod-sensitivity followed the trends described above for total biomass. These results suggest that *Gl15-TG* does not dramatically alter N uptake efficiency, such that the observed variation in plant N accumulation in these evaluations can largely be attributed to more N accumulated by larger plants, and vice versa.

One-tailed Student's T-tests with H_0 = *'no significant difference due to to the effect of the transgene'* , $\alpha = 0.05$,were performed for nitrogen content, stover dry weight, grain weight, and total biomass for the 2010 dataset (Table 2.1). There is a trend towards greater nitrogen content in *Gl15-TG* hybrids compared to their non-transgenic isolines when supplemented with N fertilizer, but this is not apparent when N is limiting. At both N levels, stover dry weight for transgenic genotypes is significantly higher than non-transgenic controls, whereas *Gl15-TG* appears to have a significant impact on grain weight only significant for the B73 x H99 background. These results may reflect the earlier maturity of FR1064 x H99 relative B73 x H99, where the impact of delayed shoot maturation due to *Gl15-TG* might be expected to be greater.

It is interesting that the T-test for difference in total biomass between the transgenic and the non-transgenic groups show so few significant differences in the 2010 dataset. As this is mostly shown in the 0 kgN/ha treatment, one possible explanation is that the B73 x H99 and FR1064 x H99 hybrid backgrounds have been selected for maximum grain production under nitrogen stress and not to allocate resources to vegetative growth. While stover biomass is significantly increased for most of the *Gl15-TG* genotypes and there is an observable increase in total biomass yield, higher grain production in the non-transgenic hybrid allowed them to reach a level of total biomass that is not significantly different from the *Gl15-TG* hybrid. FR1064 x H99 in particular is an interesting, as *Gl15-TG* did not significantly impact stover or total biomass in the 0 kgN/ha treatment, but increased both total biomass and stover significantly ($\alpha = 0.1$) when grown under high N fertility. This may be due to a higher nitrogen requirement for the FR1064 x H99 hybrid.

The Effect of Glossy15 Overexpression on Heterosis for Total Biomass

Stover dry weight, grain weight and total N content for transgenic and non-transgenic inbreds are shown in comparison with their hybrids in Table 2.2. The degree of heterosis for total biomass was estimated for two *Gl15-TG* hybrids derived from these inbred lines, and their non-transgenic isolines. Due to the near absence of grain production from *Gl15-TG* inbreds, it was difficult to estimate heterosis for grain yield. We found similar degrees of heterosis (29-39%) among the non-transgenic hybrids, which is not surprising since they share the H99 parent and FR1064 is an improved version of B73. *Gl15-TG* had opposing effects on heterosis among the two hybrids,

where it was increased in the B73 x H99 background, but suppressed in the FR1064 x H99 background. We attribute these differences to the observation that *Gl15-TG* increases total biomass in the FR1064 inbred, but reduces it in the B73 inbred, whereas there is little effect of *Gl15-TG* on biomass yields of H99.

Glossy15 as a Tool for Bioenergy Crop Breeding

Breeding crops for bioenergy relies on increases in vegetative growth that leads to higher total biomass, which makes flowering time-related genes and QTLs an attractive subject of study. Our experiments with *Glossy15* overexpression show that with the delayed timing of both vegetative and reproductive phase change, total biomass production and vegetative growth can be increased from the non-transgenic genotypes, but nitrogen response remains the same. Increasing corn stover with *Glossy15* overexpression have some advantages over the use of photoperiod-sensitive germplasm in that the transgene can be transformed into genotypes with known agronomic traits, resistance to pests and pathogens, and adaptation to local climate. The use of *Gl15-TG* to delay shoot maturation among current elite inbreds and hybrids may be more efficient than extracting this trait from unadapted tropical germplasm. There is also the possibility of future work with *Glossy15* or other genes within the shoot maturation pathway as tools to increase total plant biomass in C4 grass species being considered as bioenergy feedstocks.

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Tables

Table 2.1 Student's T-test on nitrogen content, stover dry weight, grain weight, total biomass and nitrogen utilization.

Comparison	N content	Stover DW	Grain	Total Biomass
0 kgN/ha				
B73 x H99, *Gl15-TG* versus no transgene	NS	*	**	NS
FR1064 x H99, *Gl15-TG* versus no transgene	NS	**	NS	NS
Temperate x Tropical hybrids, *Gl15-TG* versus no transgene	NS	NS	NS	NS
200 kgN/ha				
B73 x H99, *Gl15-TG* versus no transgene	*	**	**	NS
FR1064 x H99, *Gl15-TG* versus no transgene	**	*	NS	*
Temperate x Tropical hybrids, *Gl15-TG* versus no transgene	*	**	*	NS

* denotes significance at $\alpha = 0.1$
** denotes significance at $\alpha = 0.05$
NS denotes non-significance

Table 2.2 Biomass yield, partitioning, and heterosis among *Gl15-TG* and non-transgenic isoline inbred and hybrids.

Genotype	Biomass (g/plt)			% Heterosis
	Stover	Grain	Total	Total Biomass
Inbreds				
H99	33	47	94	-
H99 : 0/*GL15-TG*	68	1	76	-
H99 : *GL15-TG*/*GL15-TG*	92	0	97	-
B73	77	57	155	-
B73 : 0/*GL15-TG*	117	0	118	-
B73 : *GL15-TG*/*GL15-TG*	101	0	102	-
FR1064	53	18	77	-
FR1064 : 0/*GL15-TG*	144	2	149	-
FR1064 : *GL15-TG*/*GL15-TG*	155	0.00	155	-
Hybrids				
B73 x H99	71	86	173	39
B73 x H99 : *GL15-TG*	130	37	180	81
FR1064 x H99	51	49	110	29
FR1064 x H99 : *GL15-TG*	85	40	137	9

Figures

Figure 2.1 Biomass yields and partitioning among *Gl15-TG* hybrids and their non-transgenic isolines, grown without supplemental N in 2009. The values for total biomass are indicated by the height of green (stover biomass) and yellow (grain biomass) bars. The blue bar at right indicates plant N uptake. *Gl15-TG* dosage within a hybrid background is indicated by either none (-), hemizygous (+), or homozygous (++).

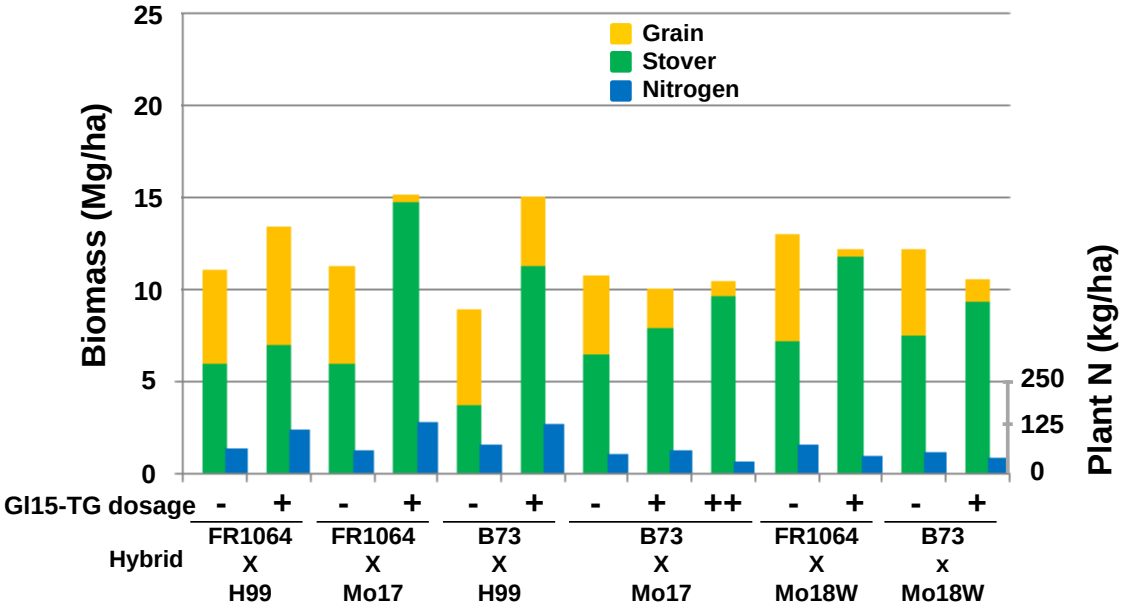


Figure 2.2 Biomass yields and partitioning among *Gl15-TG* hybrids and their non-transgenic isolines, grown with 200 kg supplemental N per hectare in 2009. The values for total biomass are indicated by the height of green (stover biomass) and yellow (grain biomass) bars. The blue bar at right indicates plant N uptake. *Gl15-TG* dosage within a hybrid background is indicated by either none (-), hemizygous (+), or homozygous (++).

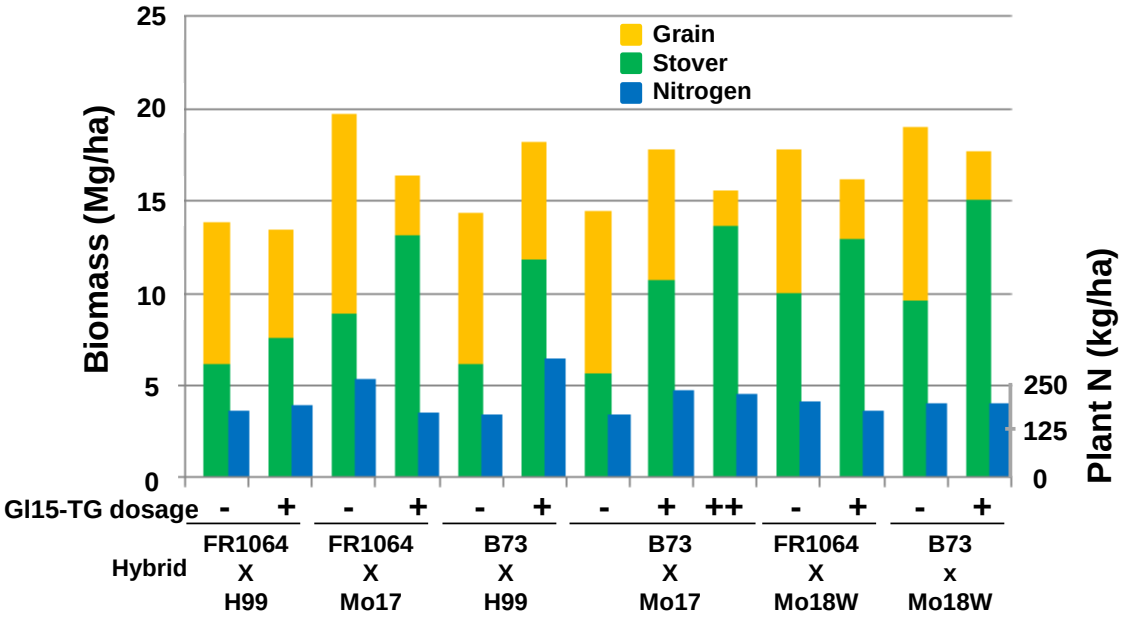


Figure 2.3 Biomass yields and partitioning among *Gl15-TG* hybrids and their non-transgenic isolines, grown without supplemental N in 2010. The values for total biomass are indicated by the height of green (stover biomass) and yellow (grain biomass) bars. The blue bar at right indicates plant N uptake. *Gl15-TG* dosage within a hybrid background is indicated by either none (-) or hemizygous (+).

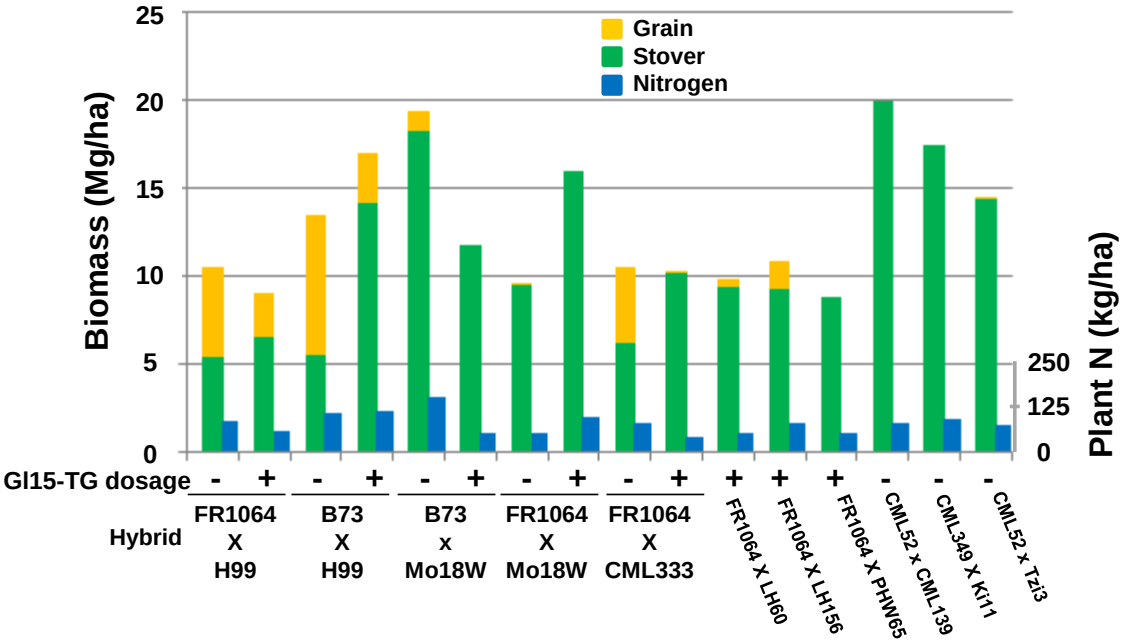


Figure 2.4 Biomass yields and partitioning among *Gl15-TG* hybrids and their non-transgenic isolines, grown with 200 kg N per hectare of supplemental N in 2010. The values for total biomass are indicated by the height of green (stover biomass) and yellow (grain biomass) bars. The blue bar at right indicates plant N uptake. *Gl15-TG* dosage within a hybrid background is indicated by either none (-) or hemizygous (+).

