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EMS induced mutations in dhurrin metabolism and their impacts on sorghum growth and development

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Head of the Department Graduate Program   Date

EMS INDUCED MUTATIONS IN DHURRIN METABOLISM AND THEIR

IMPACTS ON SORGHUM GROWTH AND DEVELOPMENT

A Thesis

Submitted to the Faculty

of

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by

Jenae L. Skelton

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of

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I would especially like to thank my parents, my fiancé, my family and friends who supported me during my graduate studies. Without their support, I might not have had the strength and motivation to keep going.

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LIST OF ABBREVIATIONS

1700 x g – 1700 times earth's gravitational acceleration

30/s – 30 Hertz

A – adenine

ACRE – Agronomy Center for Research and Education at Purdue University

ANOVA – Analysis of Variance

bp – base pairs

C - cytosine

C $\rightarrow$ T – cytosine to thymine transformation

CCI – Chlorophyll Content Index

CGs – cyanogenic glucosides

cm – centimeters

CYP – Cytochrome P450

Dhr1 – Dhurrinase1

Dhr2 – Dhurrinase-2

DIF – column heading indicating if differences were statistically significant

DN – dhurrin negative

DNA – deoxyribonucleic acid

DT – drought treatment

DW – dry weight

EMS – ethyl methanesulfonate

F_2 – filial generation 2

F_3 – filial generation 3

FW – fresh weight

g – grams

G – guanine

G → A – guanine to adenine transformation

HCN – hydrogen cyanide

HCNp – hydrogen cyanide potential

H.I. – Harvest Index

HPLC – high-performance liquid chromatography

IPA – isopropanol

KASP – Kompetitive Allele Specific PCR

kgs – kilograms

kg ha^{-1} – kilograms per hectare

liters ha^{-1} – liters per hectare

LSD – least significant difference

M – Molar

mgs – milligrams

min – minutes

ml – milliliters

mm – millimeters

mM – milliMolar

N – nitrogen

NS – means are not statistically different

nm – nanometers

PAR – photosynthetically active radiation

PCR – polymerase chain reaction

PMP – paramagnetic particles

QPCR – quantitative polymerase chain reaction

QTL – quantitative trait loci

RWC – relative water content

S – means are statistically different

Sig. – statistical significance

SLN – specific leaf nitrogen

SNP – single nucleotide polymorphism

T – thymine

TW – turgid weight

USDA – United States Department of Agriculture

VWC – volumetric water content

WT – wild-type

WW – well-watered treatment

µl – microliters

µg – micrograms

µmol s^{-1} m^{-1} – micromoles of photons per second per square meter

ABSTRACT

Skelton, Jenae L. M.S., Purdue University, August 2014. EMS Induced Mutations in Dhurrin Metabolism and Their Impacts on Sorghum Growth and Development. Professor: Mitchell Tuinstra.

Sorghum is the fifth most important grain crop in the world. It is a staple food, feed, and silage crop in many developing countries in the semi-arid tropics. One factor that can impact sorghum forage quality is dhurrin content. Dhurrin is a cyanogenic glucoside naturally produced in the plant. When tissues containing dhurrin are crushed, hydrogen cyanide (HCN) is released during dhurrin decomposition. HCN is toxic to humans and livestock. While there is natural genetic diversity for the concentration of dhurrin within sorghum lines, there have been no naturally occurring dhurrin-free genotypes identified to date.

We have identified ethyl methanesulfonate (EMS) mutant sorghum lines that have disruptions in the biosynthetic and catabolic pathways of dhurrin metabolism. The first objective of this research was to determine the causal mutation leading to the acyanogenic phenotype of EMS 2447 and EMS 5085. Using Next-Gen sequencing and SNP marker analysis, we determined that a C493Y mutation in the biosynthetic enzyme CYP79A1 was the cause of the disruption in dhurrin production. Previous studies have shown that sorghum plants with a mutation in this gene had slower seedling growth compared to wild-type plants. Our second objective was to test whether lack of dhurrin

production had any negative impacts on sorghum growth and development, as well as stay-green capacity. We designed an experiment to test the effects of the C493Y mutation using mutant and non-mutant plants from segregating F_2 families. Plots were planted in the summer of 2013. Plants were tested with the Feigl-Anger assay to determine the wild-type or acyanogenic phenotype of the F_2s. Throughout the growing season, samples were collected to determine differences in growth, including: chlorophyll content index (CCI), plant height, leaf number, biomass, grain yield, and harvest index. We discovered that the wild-type plants were nearly always slightly ahead of their acyanogenic siblings, and there was generally a 30% reduction in grain yield.

A separate study suggested that seedling dhurrin content is a predictor of post-anthesis stay-green capacity. We designed a second experiment to test the stay-green capacity of the C493Y mutant and the wild-type under well-watered and drought-stressed conditions in the greenhouse. Plants of wild-type BTx623 and acyanogenic EMS 5085 (*Cyp79A1-2*) and EMS 932 (*dhr2-1*) were planted in separate hills in the same pots. CCI was measured weekly and drought stress was imposed after anthesis. In general, the mutant lines had lower CCI than the wild-type, expecially under drought conditions. We believe this study could expand our knowledge of the function of dhurrin in sorghum and potentially may lead to development of genetic materials that could be used to create forage sorghum lines that eliminate the risk of dhurrin toxicity.

CHAPTER 1. LITERATURE REVIEW

1.1   Introduction

Grain sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop in semiarid

agricultural regions (Borrell et al., 2000a). Sorghum is produced as a food, feed, forage,

and silage crop in many different parts of the world. One factor that can affect sorghum

forage quality is dhurrin production (Hunt and Taylor, 1975; Busk and Møller, 2002;

Etuk et al., 2012). Dhurrin is a cyanogenic glucoside naturally produced in the plant.

When tissues containing dhurrin are consumed, hydrogen cyanide (HCN) is released

during dhurrin decomposition. While there is natural genetic diversity for dhurrin

production in sorghum lines, there have been no naturally-occurring, dhurrin-free

genotypes identified to date (Blomstedt et al., 2012).

Sorghum is well suited for regions that regularly experience hot temperatures and

drought stress during the growing season. Drought is a serious factor in crop yield losses

worldwide. Breeding crops for drought tolerance traits could be a viable way to alleviate

the effects of drought and climate change on crop production.

One trait for improving drought tolerance is called stay-green. It has been

associated with increased yield under drought conditions, but is also related to soil

fertility and nitrogen status of the plant itself (Borrell and Hammer, 2000; van Oosterom

et al., 2010a). Recent studies have shown that there is a positive correlation between stay-

green capacity and seedling dhurrin content (Burke et al., 2013).

In this literature review, scientific literature discussing dhurrin, its functions within sorghum, stay-green in sorghum, and the interaction between dhurrin and stay-green will be reviewed.

1.2   Dhurrin Metabolism in Sorghum

Cyanogenic glucosides are found in many plant species, including ferns, gynosperms, and angiosperms (Zagrobelny et al., 2004). Dhurrin is the cyanogenic glucoside particular to sorghum. Dhurrin is a β-glucoside derived from tyrosine. It is readily produced by the plant and stored within the leaf cells as a defense compound, which classifies it as a phytoanticipin (Zagrobelny et al., 2004; Møller 2010). Young sorghum seedlings can have up to 6% of their dry weight composed of dhurrin (Møller 2010). When sorghum leaf tissue is disrupted or damaged, for example by chewing of an herbivore, dhurrin is degraded by β-glucosidases and a hydroxynitrile lyase, releasing the byproduct HCN. For this reason, dhurrin is thought to be a defense compound against pests and herbivory (Hösel et al., 1987; Halkier and Møller, 1989; Morant et al., 2003; Zagrobelny et al., 2004; Krothapalli et al., 2013). When found in high concentrations in edible sorghum tissues, dhurrin poses a health risk to livestock due to the toxicity of HCN. Dhurrin may also serve as a nitrogen turnover and storage compound, although the mechanisms for in vivo turnover of dhurrin are not understood. Studies by McBee and Miller (1980) demonstrated that dhurrin concentration increases with increased nitrogen fertility. Jenrich et al. (2007) succeeded in performing in vitro decomposition of dhurrin which resulted in the production of 4-hydroxyphenolacetonitrile. They suggest that a new β-

glucosidase or dhurrinase in combination with a protein cofactor could be responsible for in vivo turnover of dhurrin.

1.2.1   Dhurrin Biosynthesis and Catabolism in Sorghum

There are six genes involved in the metabolism of dhurrin (Figure 1.1). Three enzymes (CYP79A1, CYP79E1, and UGT85B1) make up the anabolic pathway and transform tyrosine into dhurrin. When the leaf tissue is disrupted, two β-glucosidases and a hydroxynitrile lyase break down dhurrin, resulting in the release of HCN. Health problems caused by HCN can include respiratory failure, central nervous system dysfunction, and cardiac arrest (Etuk et al., 2012). Ruminants are capable of detoxifying HCN in the rumen and liver, but high consumption rate can overwhelm the system and can lead to health problems or death (Hunt and Taylor, 1975). Sorghum plants propagated under dryland conditions can have higher HCN potential (HCNp) than plants grown under irrigated conditions (Hunt and Taylor, 1975; O'Donnell et al., 2013). This could be due to an increase in dhurrin synthesis rate, a reduction in dhurrin turnover, or smaller plant size due to stunting. O'Donnell et al. (2013) measured plant biomass, HCNp, and dhurrin content in seedlings grown under osmotic stress induced by polyethylene glycol in hydroponic solution. Their results indicated that the increase in HCNp in stressed seedlings was in part due to stunted plant growth.
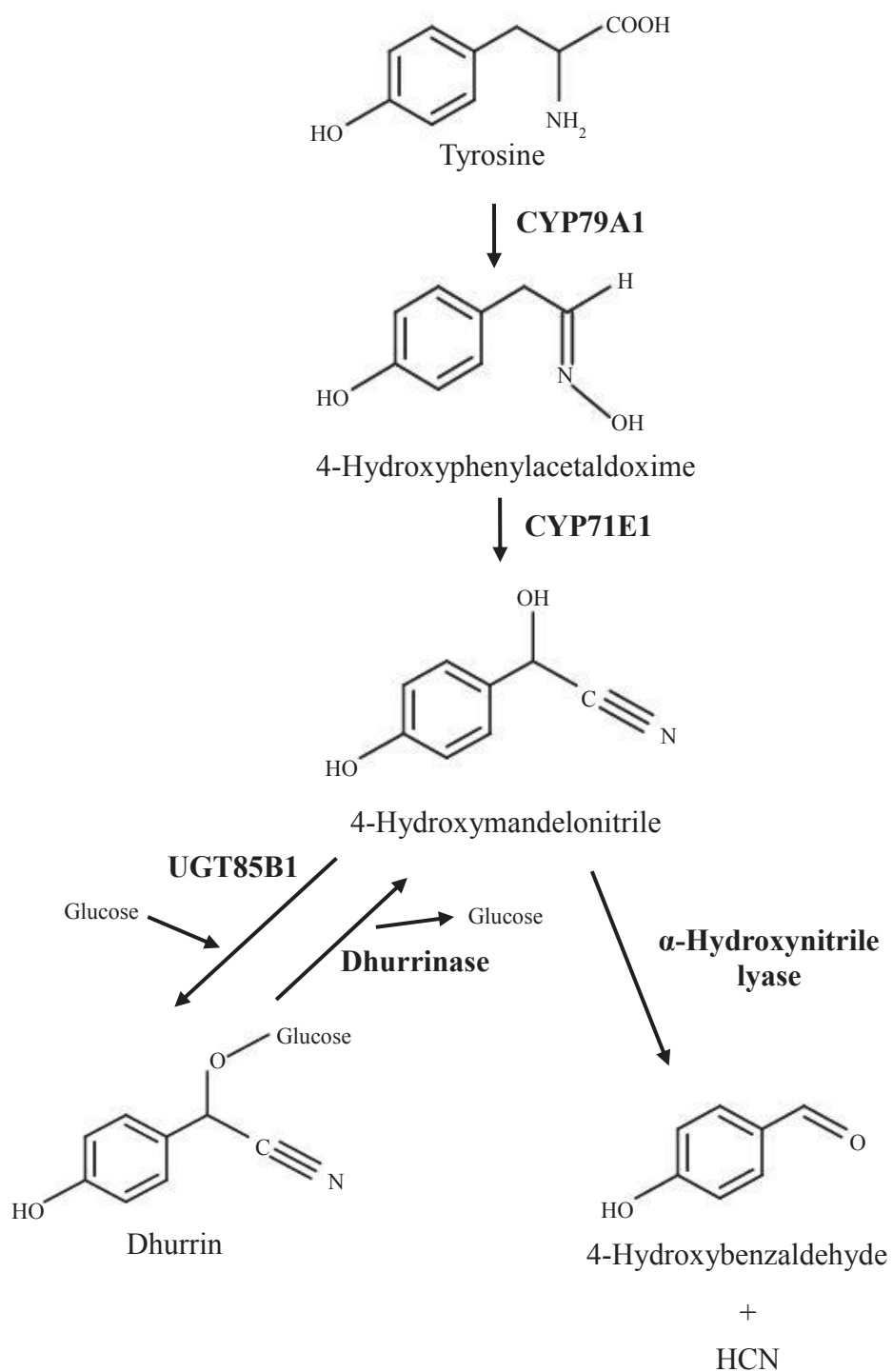
Figure 1.1 Dhurrin biosynthetic and catabolic pathway in sorghum (adapted from Krothapalli et al., 2013).

The first two enzymes involved in the biosynthesis of dhurrin are characterized as cytochrome P450 (CYP) enzymes (Halkier and Møller, 1991; Busk and Møller, 2002). CYP79A1 catalyzes the conversion of tyrosine to 4-hydroxyphenylacetaldoxime. Then CYP71E1 catalyzes the conversion of 4-hydroxyphenylacetaldoxime to 4-hydroxymandelonitrile. The final step in dhurrin biosynthesis is the conversion of 4-hydroxymandelonitrile into dhurrin by a UDP-dependent glucosyltransferase (UGT85B1) (Busk and Møller, 2002; Hansen et al., 2003).

On the catabolic side of the pathway, there are two β-glucosidases and a hydroxynitrile lyase that are involved in the degradation of dhurrin and the subsequent release of HCN. The β-glucosidases, or dhurrinases, are *dhurrinase1* and *dhurrinase2* (Hösel et al., 1987; Cicek and Esen, 1998). While both *dhurrinase1* and *dhurrinase2* are β-glucosidases, they are products of separate genes, are differentially expressed in the roots or shoots (*dhurrinase1* in the roots and stem and *dhurrinase2* in the leaves), and have different molecular weights and substrate specificity (Hösel et al., 1987). Release of HCN is initiated by the enzymatic hydrolysis of β-glucoside bonds by dhurrinase that lead to the formation of glucose and 4-hydroxymandelonitrile. The 4-hydroxymandelonitrile is further broken down by an α-hydroxynitrile lyase, leading to the release of HCN (Conn, 1979).

Cyanogenic glucosides are known to be involved in plant defense mechanisms against herbivory and pathogens. When plant cells are damaged dhurrin hydrolysis caused by β-glucosidase releases poisonous HCN gas. The enzyme and its substrate are compartmentalized within cells and come in contact when intact cell tissues are disrupted or damaged (Kojima et al., 1979; Cicek and Esen, 1998). Dhurrin is stored in vacuoles of

the epidermal cells, while dhurrinases and hydroxynitrile lyase are found in the mesophyll cells (Kojima et al., 1979). Compartmentalizing dhurrin in this manner prevents a large scale enzymatic breakdown under normal physiological conditions (Kojima et al., 1979). When cells are damaged, dhurrin is hydrolyzed by dhurrinase and leads to the production of 4-hydroxymandelonitrile, which dissociates to 4-hydroxybenzaldehyde and HCN (Cicek and Esen, 1998).

1.2.2 Dhurrin as an Herbivory Defense Compound

Several studies have reported insect feeding preferences on plants that lack cyanogenic glucosides (Morant et al., 2003; Krothapalli et al., 2013). Morant et al. (2003) transformed *Arabidopsis thaliana* plants with the anabolic pathway for dhurrin production. Their research found that flea beetles preferred dhurrin free wild-type *A. thaliana* plants over transformed plants in a free choice test. Krothapalli et al. (2013) identified an EMS mutant sorghum line that lacked cyanide release due to a single nucleotide polymorphism (SNP) mutation in dhurrinase-2. This mutation (*dhr2-1*) prevented dhurrin from degrading after cell disruption, even though dhurrin biosynthesis in the mutant line was normal. They tested feeding preference of fall army worm (*Spodoptera frugiperda*) on wild-type sorghum and the acyanogenic *dhr2-1* mutant line. The fall army worms were found to have a preference for settling and feeding on the acyanogenic mutant over the wild-type. These studies further validate the function of dhurrin as a plant defense compound.

1.2.3 Effects of Reduced Dhurrin Concentration

While dhurrin may benefit the sorghum plant by providing a defense mechanism against insect herbivory and a nitrogen storage compound, it can have detrimental effects to livestock that consume the edible plant tissues. However, aside from the toxicity issue, sorghum still has characteristics that make it a desirable forage crop including: high dry matter yield, capacity for rapid regrowth, efficient use of water due to its C4 photosynthesis, and efficient use of nitrogen in low fertility conditions (Wheeler and Mulcahy, 1989). Reducing or eliminating dhurrin production in sorghum could reduce the risk of toxicity to sorghum consumers.

While no acyanogenic mutants have been identified in natural sorghum populations, EMS mutations could lead to identification of such lines (Blomstedt et al., 2012). Krothapalli et al. (2013) identified an EMS mutant line defective in HCN release using the Feigl-Anger paper test (Feigl and Anger, 1966) and determined the causal mutation to be in *dhr2*. Blomstedt et al. (2012) identified a mutant line with a P414L mutation in CYP79A1 which caused complete disruption of the dhurrin anabolic pathway and prevented dhurrin from being synthesized. The only differences they noted between the acyanogenic mutant and the control genotype was slower growth in the seedling stage, although no data was provided. If these acyanogenic mutations can be incorporated into elite varieties without negatively affecting yield and other forage qualities, this could lead to sorghum genotypes without the risk of HCN toxicity and decreased concern for producers of dual-purpose sorghum.

1.3   Stay-Green in Sorghum

The stay-green trait has been associated with late-season drought tolerance in sorghum, maize, and other crop species. The stay-green trait is defined as extended foliar greenness during the grain filling period, or the retention of green leaf area under post-anthesis drought stress (Borrell et al., 2001). By retaining active photosynthetic capabilities, drought-stressed plants with stay-green capacity have less yield loss compared to drought-stressed plants that senesce at normal rates (Thomas and Smart, 1993; Borrell et al., 2000b; Borrell et al., 2001). Increased stay-green expression under drought stress has also been shown to reduce susceptibility to stalk-rotting pathogens and lodging when compared to normally senescing lines under the same conditions, which also contributes to reduced yield losses under drought conditions (Thomas and Smart, 1993; Borrell et al., 2000b).

Thomas and Smart (1993) classified the different types of stay-green traits (Table 1.1). Plants with functional stay-green traits retain active photosynthesis throughout the grain filling period, and can exhibit numerous forms of expression. Type A stay-green trait is defined as having delayed initiation of leaf senescence then followed by a normal rate of senescence. Type B stay-green plants initiate senescence at the same time as their senescent counterparts, but the rate of senescence is slower than a normally senescing plant. Alternately, plants with cosmetic stay-green traits retain green chlorophyll pigments but lose functional photosynthetic capabilities. There are three different types of cosmetic stay-green traits in plants. Type C stay-green plants retain chlorophyll pigments but lose their photosynthetic capability. Hauck et al. (1997) identified a mutant of *Festuca pratensis* that retained chlorophyll content after full leaf expansion but did not

increase or maintain photosynthetic rate or CO_2 assimilation, identifying this mutant as

having the Type C stay-green trait. The Type D stay-green trait accounts for dead plants

that maintain their green color, such as herbarium specimens or frozen vegetables. Plants

with the Type E, accounts for plants that have greater initial greenness and essentially

have more pigments than other related plants, which accounts for their apparent extra

greenness.

Table 1.1 Summary of stay-green traits according to their classification as functional or
cosmetic and a description of distinguishing characteristics.

Type	Classification	Characteristics
A	Functional	Delayed onset of senescence
B	Functional	Decreased rate of senescence
C	Cosmetic	Chlorophyll pigments retained but photosynthetic capacity lost
D	Cosmetic	Dead plants retaining greenness after preservation
E	Cosmetic	Higher initial greenness level compared to related plants

These classifications can be useful in understanding the underlying physiological

or genetic differences in stay-green phenotypes. However, plants can have combinations

of stay-green traits. There are also alternative methods that can delay yellowing of leaves

(Thomas and Howarth, 2000). In mature leaves, shading can help extend the duration of

green leaf area. Removal of the shoot above a yellowing leaf can stop or even reverse

senescence. Chemicals with various modes of action from growth regulators to metabolic

inhibitors can halt chlorophyll degradation (Thomas and Howarth, 2000). While these

processes may alter the biochemical or physiological processes involved in stay-green

traits, they are influenced by environmental factors or cultural practices and would not be

heritable traits in the offspring. Only by manipulating the genes involved in the regulation of stay-green traits would the resulting phenotype be passed on to the next generation.

How is this extended greenness with retained photosynthetic capabilities achieved? At the cellular level, plants with functional stay-green traits demonstrated delayed degradation of chloroplast-associated proteins and maintained photosynthesis longer during grain-fill (Borrell et al., 2001). When analyzing stay-green expression in the entire leaf, the durability of photosynthetic and stay-green capacity demonstrated a close relationship with the nitrogen status of the plant, especially the specific leaf nitrogen (SLN), calculated as leaf nitrogen (N) per unit leaf area, at the initiation of senescence (van Oosterom et al., 2010a).

Previous studies identified several quantitative trait loci associated with drought tolerance and stay-green expression in sorghum (Tuinstra et al., 1997; Xu et al., 2000; Kebede et al., 2001; Sanchez et al., 2002; Harris et al., 2007). These QTL are referred to as *Stg1*, *Stg2*, *Stg3* and *Stg4*. These studies found that the first three QTL could account for over 40% of the phenotypic variation of amount of leaf senescence and chlorophyll content in a study of sorghum plants under post-flowering drought stress.

Borrell et al. (2000a and 2000b) conducted a study to understand the effects of different watering regimes on sorghum hybrids differing in senescence and how closely the visual rating of the stay-green trait was associated with green leaf area at maturity. They created nine hybrids from parents with two different sources of the stay-green trait. The hybrids varied from senescent to incomplete to stay-green. They used three different water regimes (no deficit, post-flowering deficit, and terminal deficit) to simulate drought conditions on all nine hybrids. At the end of the study, the stay-green hybrids produced

47% more biomass than the senescent hybrids under terminal drought stress. During grain fill, the stem dry mass remained constant for the stay-green hybrid, while stem mass declined in the second half of the grain fill period for the intermediate hybrid and was constantly low for the senescent hybrid. The panicle mass during the first half of grain fill was relatively the same amongst all hybrids, while the stay-green hybrid produced more during the second half of grain fill than both the intermediate and senescent hybrids. While yield for the senescent hybrids declined with an increase in water deficit, the stay-green hybrids maintained yield across the water deficit treatments. Their results showed no differences in yield between 8 of the 9 hybrids under the fully irrigated treatment. Since the hybrids varied in their rate of leaf senescence, this would indicate that the stay-green trait is not restricting under ideal conditions, yet it can improve yield in drought conditions over senescent hybrids.

Extended foliar greenness during grain fill has been shown to increase yield under drought conditions, as mentioned previously. Along with disease and lodging resistance benefits, there are also potential benefits for improving feed quality of dual-purpose sorghums, especially in the semi-arid tropics (Borrell et al., 2000b; Zerbini and Thomas, 2003). The greater functional leaf area during grain fill contributes to the increased accumulation of soluble sugars which reduces plant dependence on stored assimilates to fill the grain. This increase in stem sugars results in improved quality of the stover for use in animal feed. If this improved nutrition could be combined with lower lignin content as found in the brown mid-rib type sorghums, there could be potential for even further improvement in sorghums used as livestock feed (Sattler et al., 2010).

1.3.1 Relationship between Stay-Green Capacity and the Nitrogen Status of the Plant

There are two sources of nitrogen for the plant to use in producing seed. One source is the soil available nitrogen absorbed through the roots. The other source is nitrogen stored in and remobilized from the other plant tissues. Both of these sources can affect the consistency of stay-green expression (Borrell and Hammer 2000; van Oosterom et al., 2010b). However, remobilized nitrogen in the plant is the key source when plants are grown in nitrogen-limited or drought-prone environments (Borrell et al., 2001). When senescence begins, plants cease to produce amino acids and instead, existing proteins are degraded and the resulting amino acids are translocated out of the leaf (Borrell and Hammer, 2000). The characteristic yellowing associated with senescence is due to the degradation of leaf proteins in chlorophyll pigments which results in color loss when they are broken down. While genetic components can increase stay-green expression, it can also be attributed to the balance between N demand for grain fill and the available N supply (Borrell and Hammer, 2000; Borrell et al., 2001).

In sorghum under drought stress, yield was positively correlated with green leaf area at maturity in Australia and at mid-grain fill in India (Borrell and Hammer, 2000; Borrell et al., 2001). Borrell et al. (2001) reported that higher SLN in stay-green hybrids when compared to intermediate and senescent hybrids at anthesis was an indicator that the stay-green hybrids had a higher N concentration even before the stay-green phenotype was visibly evident. They also noted that SLN and leaf area index were correlated at maturity. This could indicate that there is a certain critical level of SLN under which senescence initiates.

1.3.2 Relationship between Dhurrin and Stay-Green

Burke et al. (2013) found that high dhurrin content in seedlings was associated with increased stay-green capacity at maturity. As reviewed above, dhurrin is believed to be an N storage compound (although the mechanism has not been determined), and may influence the stay-green trait through altered N status of the plant. Therefore, it would be reasonable to speculate that increased dhurrin content would mean an improved N status in the plant, which could then lead to increased stay-green capacity later in the life of the plant. However, dhurrin content and HCNp decrease in the plant as it matures, possibly due to remobilization or cessation of dhurrin synthesis (McBee and Miller, 1980; Gorz et al., 1985; Busk and Møller, 2002).

The objectives of this research seek to 1) identify and characterize candidate genetic mutations in a sorghum mutant that does not accumulate dhurrin, 2) determine the effects of EMS-induced dhurrin loss on sorghum growth and development, and 3) determine the relationship between dhurrin concentration and the stay-green trait in sorghum.

CHAPTER 2. GENETIC ANALYSIS OF EMS INDUCED DHURRIN MUTANTS

2.1   Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop plant in semiarid

agricultural regions (Borrell et al., 2000a). It is produced as a food, feed, forage, and

silage crop in many different parts of the world. Dhurrin is a cyanogenic glucoside

naturally produced in sorghum (Hunt and Taylor, 1975; Busk and Møller, 2002; Etuk et

al., 2012). When tissues containing dhurrin are macerated, hydrogen cyanide (HCN) is

released during dhurrin decomposition.

Cyanogenic glucosides are found in many plant species, including ferns,

gynosperms, and angiosperms (Zagrobelny et al., 2004). Dhurrin is the cyanogenic

glucoside unique to sorghum. Dhurrin is a β-glucoside derived from tyrosine. The first

two enzymes involved in the biosynthesis of dhurrin are characterized as cytochrome

P450 (CYP) enzymes (Halkier and Møller, 1991; Busk and Møller, 2002). CYP79A1

catalyzes the conversion of tyrosine to 4-hydroxyphenylacetaldoxime. Then CYP71E1

converts 4-hydroxyphenylacetaldoxime to 4-hydroxymandelonitrile. The final step in

dhurrin biosynthesis is the conversion of 4-hydroxymandelonitrile into dhurrin by a

UDP-dependent glucosyltransferase (UGT85B1) (Busk and Møller, 2002; Hansen et al.,

2003). Two β-glucosidases and a hydroxynitrile lyase are involved in the degradation of

dhurrin and the subsequent release of HCN. The β-glucosidases, or dhurrinases, are *Dhr1*

and *Dhr2* (Cicek and Esen, 1998). While both *Dhr1* and *Dhr2* are β-glucosidases, they

are products of separate genes, are differentially expressed in the roots or shoots (*Dhr1* in the roots and stem and *Dhr2* in the leaves), and have different molecular weights and substrate specificity (Hösel et al., 1987). Release of HCN is initiated by the enzymatic hydrolysis of β-glucoside bonds by dhurrinase that lead to the formation of glucose and α-hydroxynitrile. The α-hydroxynitrile is further broken down, leading to the release of HCN (Conn, 1979).

Dhurrin is readily produced by the plant and stored within the leaf cells as a defense compound, which classifies it as a phytoanticipin (Zagrobelny et al., 2004; Møller, 2010). Young sorghum seedlings can have up to 6% of their dry weight composed of dhurrin (Møller, 2010). The enzyme and its substrate are compartmentalized within the cell and come in contact when the intact cell tissues are disrupted or damaged (Kojima et al., 1979; Cicek and Esen, 1998). The dhurrin is stored in the vacuoles of the epidermal cells, while the dhurrinases and hydroxynitrile lyase are found in the mesophyll cells (Kojima et al., 1979). Compartmentalizing dhurrin in this manner prevents a large scale enzymatic breakdown under normal physiological conditions (Kojima et al., 1979). When sorghum leaf tissue is disrupted or damaged, for example by chewing of an herbivore, dhurrin is degraded by β-glucosidases and a hydroxynitrile lyase, releasing the byproduct HCN. For this reason, dhurrin is believed to be a defense compound against pests and herbivory (Hösel et al., 1987; Halkier and Møller, 1989; Morant et al., 2003, Zagrobelny et al., 2004; Krothapalli et al., 2013). When dhurrin is found in high concentrations in edible sorghum tissues, it poses a health risk to livestock due to the toxicity of HCN. Health problems caused by HCN can include respiratory failure, central nervous system dysfunction, and cardiac arrest (Etuk et al., 2012).

Ruminants are capable of detoxifying HCN in the rumen and liver, but high consumption rate can overwhelm the system and ultimately lead to health problems or death (Hunt and Taylor, 1975).

Environmental conditions can have a large impact on dhurrin content of sorghum tissues. Sorghum plants grown under dryland conditions produce more dhurrin than plants grown under irrigation (Hunt and Taylor, 1975). O'Donnell et al. (2013) grew sorghum hydroponically using polyethylene glycol (PEG) to induce osmotic stress to simulate drought conditions. They determined that increased dhurrin concentration after drought stress is in part due to stunted growth of stressed plants.

Studies by McBee and Miller (1980) demonstrated that dhurrin concentration increases with increased nitrogen fertility. Jenrich et al. (2007) succeeded in performing in vitro decomposition of dhurrin, which resulted in the production of 4-hydroxyphenolacetonitrile. They suggested that a new β-glucosidase or dhurrinase in combination with a protein cofactor could be responsible for in vivo turnover of dhurrin.

Several studies have reported insect feeding preferences on plants that lack cyanogenic glucosides (Morant et al., 2003; Krothapalli et al., 2013). Morant et al. (2003) transformed *Arabidopsis thaliana* plants with the anabolic pathway for dhurrin production. Their research found that flea beetles preferred the dhurrin-free wild-type *A. thaliana* plants over the transformed plants in a free choice test. Krothapalli et al. (2013) identified an EMS mutant sorghum line that lacked cyanide release due to a single nucleotide polymorphism (SNP) mutation in *dhurrinase2* (*Dhr2-1*). This mutation prevented dhurrin from degrading after cell disruption, even though dhurrin biosynthesis in the mutant line was normal. They tested the feeding preference of fall army worm

(*Spodoptera frugiperda*) on wild-type sorghum and the acyanogenic *Dhr2-1* mutant line. The fall army worms were found to have a preference for settling and feeding on the acyanogenic mutant over the wild-type. These studies further validate the function of dhurrin as a plant defense compound.

Sorghum still has many characteristics that make it a desirable forage crop including high dry matter yield, capacity for rapid regrowth, efficient use of water due to its C4 nature, and efficient use of nitrogen in low fertility conditions (Wheeler and Mulcahy, 1989). Reducing or eliminating dhurrin production in sorghum could reduce the risk of toxicity to sorghum consumers.

Genetic studies have demonstrated that there is considerable quantitative variation in dhurrin content among sorghum lines but no acyanogenic mutants have been identified in the standing genetic variation of sorghum (Hogg and Ahlgren, 1943; Gorz et al., 1977; Gorz et al., 1986; Burke et al., 2013). Krothapalli et al. (2013) identified a *Dhr2-1* mutant line defective in HCN release using a Feigl-Anger paper test of sorghum seedlings (Feigl and Anger, 1966). Blomstedt et al. (2012) identified a mutant line with a P414L mutation in CYP79A1, which caused complete disruption of the dhurrin anabolic pathway and prevented dhurrin from being synthesized but had negative effects on plant biomass accumulation.

The objective of this study was to 1) characterize a newly discovered acyanogenic mutant of sorghum to identify the mutation responsible for this phenotype and 2) quantify how the mutation affects dhurrin concentration. If these acyanogenic mutations can be incorporated into elite varieties without negatively affecting yield and other forage

qualities, this could lead to sorghum genotypes without the risk of HCN toxicity and

decreased concern for producers of dual purpose sorghum.

2.2   Materials and Methods

2.2.1   Identification of Dhurrin Mutants

2.2.1.1   EMS Sorghum Population Development

An EMS mutant population of sorghum lines was developed at Purdue University as

described by Krothapalli et al. (2013). In summary, 1.5 kg seed samples of Tx623 were

treated with 25, 35, or 45 mM EMS for approximately 8 hours then rinsed thoroughly

with water. The mutagenized seeds were planted in fields at the Purdue University

Agronomy Center for Research and Education (ACRE) in the summer of 2009.

Approximately 12,000 fertile plants were harvested from the 45 mM EMS seed treatment

and self-pollinated for two generations in 2010 and 2011 to produce M3 seeds.

2.2.1.2   Genetic Screen for HCN Production

The EMS population was screened for variation in HCN release using the Feigl–Anger

paper assay as previously described (Feigl and Anger, 1966; Krothapalli et al., 2013).

The Fiegl-Anger assay detects the presence of HCN, a byproduct of dhurrin

decomposition. Since HCN is not produced from sorghum tissues that do not contain

dhurrin, this assay is an efficient method for identifying dhurrin metabolism mutants. The

Feigl-Anger assay was conducted with Whatman filter paper treated with copper

ethylacetoacetate (Alfa Aesar) and tetra base 4,4-methylenebis (N,N-dimethylaniline)

(Sigma-Aldrich) to detect release of HCN from sorghum tissues. In a 250 ml bottle, 1g of

copper ethylacetoacetate (Alfa Aesar) was added to 100 ml of chloroform, capped loosely, and placed on a stir plate heated to 95 ºC until dissolved. In a separate 250 ml bottle, 1g of tetra base 4,4-methylenebis (N,N-dimethylaniline) (Sigma-Aldrich) was added to 100 ml of chloroform, covered, and placed on a stir plate until dissolved. Under the fume hood, the tetra base solution was combined with an equal volume of copper ethylacetoacetate solution and gently mixed. Approximately 6 ml of the combined solution was placed in a rectangular glass container and sheets (11x14 cm size) of Whatman 3MM paper were placed in the container and wetted on both sides with the solution. The damp paper was placed on a drying rack until completely dry, then stored in a plastic storage bag in the refrigerator until needed.

Plants were sampled for HCN release at the 2- to 3-leaf stage. A 2.5 cm section of the youngest leaf tip was sampled using scissors and gently placed into a well of a 96-well polystyrene flat-bottomed microtiter plate using forceps. Once all samples were collected, a lid was placed on the plate and secured with tape, then the plate was placed at -80 ºC overnight to freeze the tissues. After freezing, a sheet of treated Whatman paper was placed on top of the plate wells and covered with a plate lid. The plate was placed in an incubator at 35 ºC to thaw the tissue. Weights were placed on top of the plate lid to maintain a tight seal to prevent HCN leakage between plate wells. The plate was incubated for 10 to 15 minutes until blue spots were visible on the Whatman paper. The plate was removed from the incubator and the reaction for each plate well was recorded. Wells that contained wild-type tissue released HCN that reacted with the paper, producing a blue spot on the paper (Figure 2.1). These wells were recorded as "blue". Tissues that lacked HCN production (acyanogenic mutant type) failed to react with the

paper and no color change was observed, thus these wells were labeled as "white", the

original color of the treated Whatman paper (Figure 2.1).
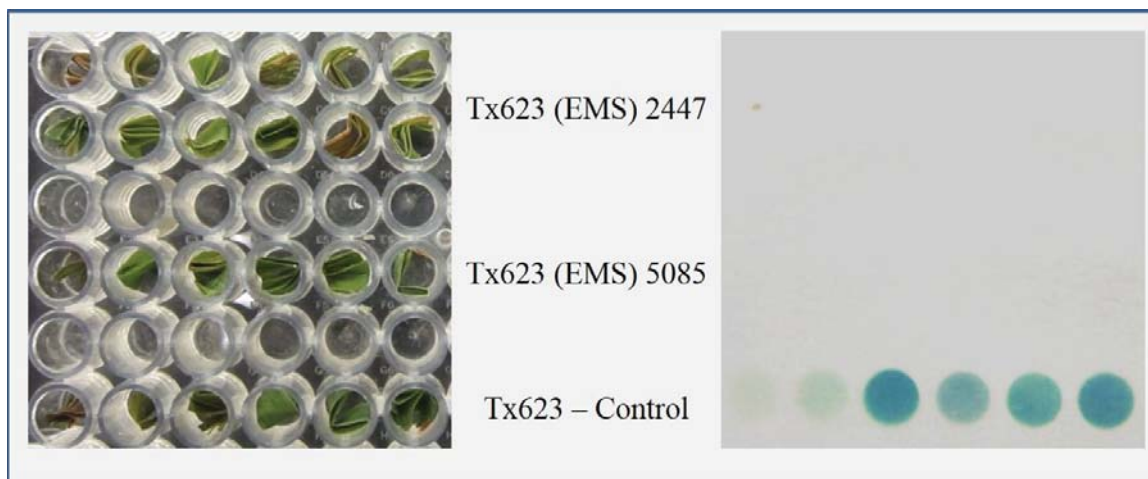
Figure 2.1 Feigl-Anger assay comparing tissue samples from the acyanogenic mutant
lines EMS 2447 and EMS 5085 to the wild-type Tx623. Note the lack of color change to
the paper for the acyanogenic lines.

2.2.2   Identification of Causal Mutations

2.2.2.1   DNA Extraction and Sequencing

The causal mutations for the acyanogenic EMS 2447 and EMS 5085 mutants were

identified by whole-genome resequencing. DNA was isolated from homozygous mutant

plants. Young leaf tissue (100-200 mg) was ground in a 1.5 ml microcentrifuge tube in

liquid nitrogen using a metal rotary pestle and electric grinder. In each tube, 700 μl of

extraction buffer [7 M urea, 50 mM Tris-HCl pH 7.5, 0.3 M NaCl, and 20 mM EDTA pH

8.0, 2% (w/v) Sarkosyl] was added, then 700 μl of phenol:chloroform:isoamyl alcohol

(25:24:1) with 10 mM Tris 8.0 (Sigma Aldrich). The samples were thoroughly mixed and

then centrifuged at 17950 x g for 10 min. The aqueous phase was then transferred to a

clean 1.5 ml microcentrifuge tube and the DNA was precipitated by adding 1/10 volume

sodium acetate (pH 5) followed by an equal volume of cold isopropanol. DNA was

pelleted by centrifugation at 17,950 x g for 10 min, then washed with 70% ethanol and

resuspended in 200 μl of distilled water.

DNA samples were submitted to the Purdue University Genomics Core Facility

(West Lafayette, IN) for whole genome resequencing. Sequencing was performed using

an Illumina HiScanSQ instrument (Illumina, San Diego). Approximately 20 million 100-

bp paired-end sequencing reads resulted in 17-fold genomic coverage when aligned to the

BTx623 reference genome sequence (Paterson et al. 2009). Single nucleotide

polymorphisms (SNPs) were identified using the Illumina CASAVA pipeline (Software

version 1.7.0, 2011).

2.2.2.2 Bioinformatics

A bioinformatics workflow developed at Purdue University was used to identify the

causal mutation in the DNA sequence (Krothapalli et al., 2013; personal communication

with Elizabeth Buescher, Department of Horticulture and Landscape Architecture,

Purdue University). The SNPs in EMS 2447 and EMS 5085 EMS mutant lines identified

using the Illumina CASAVA pipeline were provided in separate text files for each

chromosome. These files were uploaded into Galaxy (usegalaxy.org), "an open-sourced,

web-based platform for data intensive biomedical research" (Giardine et al., 2005;

Blankenburg et al., 2010; Goecks et al., 2010). Once the individual files were uploaded

into the program, a new column was added to the files using the "Text Manipulation"

function. For each file, a column containing the number of the corresponding

chromosome was added before the files were concatenated into one larger, complete

dataset. The large dataset was filtered to identify C → T or G → A SNPs using separate

commands in the "Filter and Sort" function. It is known that EMS induces these

particular mutations (Tessman et al., 1964). The resulting files containing only the C → T

or G → A SNPs were concatenated into a single file.

The next step was to determine if any of the SNPs were located within any of the

six known genes in the dhurrin metabolic pathways (Krothapalli et al., 2013). The

collection of exons within the sorghum genome (Paterson et al., 2009) was obtained from

Phytozome (www.phytozome.net). This annotated exon file was also uploaded into

Galaxy. The program was used to compare all C → T or G → A SNPs from the initial

file with the exon regions in the genome file. The resulting output provided information

including which SNP was detected at each locus and on which chromosome it was

located. The file was reviewed visually and compared to the sequences from the known

dhurrin pathway genes (Figure 1.1) to determine if any EMS induced SNPs were

identified.

2.2.2.3   <u>Co-Segregation Analysis</u>

Co-segregation analyses were used to test linkage between SNPs in candidate genes and

the acyanogenic mutant phenotype in EMS 2447. An F_2 population was made from a

cross between BTx623 and EMS 2447. This population was screened using the Feigl-

Anger assay to identify acyanogenic and wild-type plants. A set of 20 wild-type and

acyanogenic mutant F_2 plants was sampled for DNA extraction. A Harris Uni-Core

(Shunderson Communications, Inc.) 6.00 mm punch and Harris self-healing cutting mat were used to take leaf tissue samples. Five punches from each plant were sampled midway between the leaf base and tip. Samples were placed into microcentrifuge tubes. The samples were stored on ice until returned to the lab where they were stored at -80 °C.

DNA was extracted for PCR and marker analysis using the Wizard Magnetic 96 DNA Plant System (Promega) kit. Tissue was transferred to individual round bottom tubes containing a single 5 mm stainless steel grinding bead and placed in a 96 well latch rack. Each tube received 300 μl of Lysis Buffer A from the kit. A cap mat was used to seal the tubes. Then the rack was placed in a TissueLyser (Qiagen) machine using a 96 well plate adapter. The tissue was ground for three minutes at a frequency of 30/s, then the rack was inverted and ground for an additional three minutes at the same frequency. The plate rack was removed from the TissueLyser and centrifuged at 1700 x g for five minutes, then rotated and centrifuged at the same rate for an additional five minutes.

For each sample, 125 μl of the supernatant was transferred to a 96-well deep well plate. Each sample received 60 μl of the MagneSil PMPs/Lysis Buffer B solution and incubated at room temperature for five minutes. The plate was then placed on a MagnaBot 96 Magnetic Separation Device (Promega) for one minute to allow the magnetic particles to settle and the liquid fraction was removed by pipetting. The plate was removed from the magnetic device and each sample was washed twice using 150 μl of the wash solution, a solution prepared by adding 20 ml of ethanol and 20 ml of isopropanol to the wash buffer provided in the kit. After the last wash, the plate was placed back on the magnetic device, the liquid was removed, and the particles were allowed to dry at room temperature for five minutes. Each sample was resuspended in 50

µl of nuclease-free water and the MagneSil PMPs were re-suspended by pipetting then incubated at room temperature for an additional five minutes. The plate was placed back on the magnetic device for one minute and the liquid was transferred to a fresh 96-well plate for storage at -20 ºC until needed for marker analysis.

A Kompetitive Allele Specific PCR (KASP) genotyping assay was used to assess co-segregation between SNPs in candidate genes and the acyanogenic mutant phenotype. Allele-specific KASP primers were developed as suggested by LGC Genomics for a candidate mutation in CYP79A1 based on the candidate gene sequence with SNP shown in square brackets:

GCCTTCCGAAGAGCATGACGCTCATGGCGGTGCCGAGCGACGCGGCGATG[C/T]AGCCGCGGCGGCCGGTGCTGAAGGAGATGAACCGCAGGTCGTTCTCGGTG.

The KASPar Assay developed for this SNP was used to genotype the segregating F$_2$ family. In a 2 ml microcentrifuge tube, 4 µl per sample of KASP Master Mix and 0.11 µl of the designed KASP Assay Mix were gently mixed. After mixing, 4 µl of the reaction mixture was aliquotted to individual wells in a fully skirted 384-well PCR plate. A volume of 4 µl of DNA was added to each well according to their order in the DNA storage plate. Once all samples were placed in the plate, the plate was sealed with a QPCR adhesive seal. The plate was centrifuged briefly to bring all the components to the bottom of the wells to thoroughly mix the components. The sealed plate was placed in a LightCycler 480 Real-Time PCR System (Hoffman-La Roche Ltd.). The PCR program consisted of one pre-incubation step heating the plate up to 94 ºC, then 39 amplification cycles of 94 ºC for ten seconds and 60 ºC for one minute, followed by a cooling step at 35 ºC for 30 seconds to complete the program. The plate was then removed from the

machine and allowed to cool to room temperature. The plate was placed back in the machine for the read program, which analyzes the fluorescence given off by the allele specific primers and assigns an allele genotype (wild-type, mutant-type, or heterozygous) to each well of the plate.

2.2.3   Dhurrin Content of EMS mutants

EMS 2447, Tx623, and a Tx623 x EMS 2447 F_2 population were evaluated for differences in dhurrin content. Field plots were planted at Purdue University's Agronomy Center for Research and Education (ACRE) in West Lafayette, IN. In the fall prior to planting, 340 kg/ha of KCl was applied after the previous crop had been removed. Three weeks later the field was worked with a chisel. In the spring, one month prior to planting, 170 kg/ha of anhydrous ammonia was applied. Plots were planted at ACRE on June 5th, 2013. Weeds were controlled after planting by treating plots with Bicep (Syngenta Crop Protection, Inc.) at 3.784 liters/ha and Roundup (Monsanto Company) at 1.75 liters/ha with ammonium sulfate to treat hard water at a rate of 0.95 liters per 378.5 liters of water, then by hoeing later in the season.

Approximately 200 F_2 seeds were planted in a 60 m x 0.76 m plot at a rate of 4 plants m^{-2}. The Feigl-Anger assay was used to identify the wild-type and mutant type F_2 plants as described above. Plants were grown to the V4 stage and a Harris Uni-Core (Shunderson Communications, Inc.) 6.00 mm punch and Harris self-healing cutting mat were used to take leaf tissue samples from a set of 47 wild-type plants and 47 acyanogenic F_2 plants and 4 replications of each parent line. Five punches from each plant were sampled midway between the leaf base and tip. Samples were placed into

microcentrifuge tubes and stored on ice until returned to the lab where they were stored at

-20 °C. The tissue samples were shipped on dry ice to Dr. John Burke at the USDA

Cropping Systems Laboratory in Lubbock, TX for analysis of dhurrin content using the

HPLC protocol described by Burke et al. (2013). Eight tissue samples were also collected

from B73 maize grown in the same field to determine the base-line HPLC values, since

maize does not produce dhurrin. The resulting data was statistically analyzed using

Statistical Analysis Software (SAS Institute Inc.). The 47 F_2 samples were averaged for

each plot then compared to the means of the parents and the maize check using the GLM

procedure and Tukey's studentized range test to determine if differences between each

pair of means were statistically significant.

2.3   Results

2.3.1   Identification of Acyanogenic Mutants

The EMS population described in Section 2.2.1.1 was screened for HCN production

using the Feigl-Anger assay. This assay resulted in the identification of acyanogenic

mutant plants within the families EMS 2447 and EMS 5085 (Fig. 2.1, Section 2.2.1.2).

Individual mutant plants from EMS 2447 and EMS 5085 that did not produce HCN were

transplanted to larger pots. Preliminary observations showed that the mutant plants grew

normally and showed no visible differences in relative greenness. These plants were self-

pollinated and grown to physiological maturity for seed production. Several of the M_2

families segregating for albinism were sampled using Feigl-Anger assay to determine if

HCN production differed between the albino and green siblings from the same family.

The results indicated that albinism did not affect HCN release and therefore dhurrin

production. Since these plants are unable to normally photosynthesize due to lack of chlorophyll pigments, perhaps the dhurrin is synthesized from precursors stored and released from the endosperm reserves.

2.3.2   Candidate SNPs Identified

DNA sequencing and bioinformatics analyses of EMS 2447 identified a C → T transformation at base position 1,132,232 on chromosome 1 within the first gene of the dhurrin biosynthetic pathway, CYP79A1, a cytochrome P-450. Upon further study, it was determined that another HCN-free mutant, EMS 5085, also contained the same CYP79A1 mutation as EMS 2447. When the sequence was analyzed in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), it was revealed that the SNP causes a missense mutation in the protein changing a cysteine to tyrosine (C493Y) as shown in Figure 2.2.

```
EMS 2447   MATMEVEAAAATVLAAPLLSSSAILKLLLFVVTLSYLARALRRPRKSTTKCSSTTCASPP
BTx623     MATMEVEAAAATVLAAPLLSSSAILKLLLFVVTLSYLARALRRPRKSTTKCSSTTCASPP

EMS 2447   AGVGNPPLPPGPVPWPVVGNLPEMLLNKPAFRWIHQMMREMGTDIACVKLGGVHVVSITC
BTx623     AGVGNPPLPPGPVPWPVVGNLPEMLLNKPAFRWIHQMMREMGTDIACVKLGGVHVVSITC

EMS 2447   PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH
BTx623     PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH

EMS 2447   AWLHDKRTDEADNLTRYVYNLATKAATGDVAVDVRHVARHYCGNVIRRLMFNRRYFGEPQ
BTx623     AWLHDKRTDEADNLTRYVYNLATKAATGDVAVDVRHVARHYCGNVIRRLMFNRRYFGEPQ

EMS 2447   ADGGPGPMEVLHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANVAVNRLHDT
BTx623     ADGGPGPMEVLHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANVAVNRLHDT

EMS 2447   VIDDRWRQWKSGERQEMEDFLDVLITLKDAQGNPLLTIEEVKAQSQ
BTx623     VIDDRWRQWKSGERQEMEDFLDVLITLKDAQGNPLLTIEEVKAQSQ

EMS 2447   DITFAAVDNPSNAVEWALAEMVNNPEVMAKAMEELDRVVGRERLVQESDIPKLNYVKA
BTx623     DITFAAVDNPSNAVEWALAEMVNNPEVMAKAMEELDRVVGRERLVQESDIPKLNYVKA

EMS 2447   CIREAFRLHPVAPFNVPHVALADTTIAGYRVPKGSHVILSRTGLGRNPRVWDEPLRFYPD
BTx623     CIREAFRLHPVAPFNVPHVALADTTIAGYRVPKGSHVILSRTGLGRNPRVWDEPLRFYPD

EMS 2447   RHLATAASDVALTENDLRFISFSTGRRGYIAASLGTAMSVMLFGRLLQGFTWSKPAGVEA

BTx623     RHLATAASDVALTENDLRFISFSTGRRGCIAASLGTAMSVMLFGRLLQGFTWSKPAGVEA

EMS 2447   VDLSESKSDTFMATPLVLHAEPRLPAHLYPSISI
BTx623     VDLSESKSDTFMATPLVLHAEPRLPAHLYPSISI
```

Figure 2.2 Amino acid sequence for EMS 2447 compared to the reference genome BTx623 showing the C493Y change (enlarged and highlighted in green) created by the causal SNP mutation.

2.3.3 Co-Segregation of Mutation with KASP Markers

Allele specific KASP markers were used to evaluate whether the C493Y mutation in CYP79A1 co-segregated with the acyanogenic phenotype. The KASP marker clearly differentiated the wild-type allele in BTx623 and the mutant allele in EMS 2447 (Table 2.1). Genetic analyses of 20 segregating F_2 plants indicated the F_2 population segregated into three genotypic classes and the marker for the C493Y mutation derived from EMS 2447 co-segregated perfectly with the acyanogenic mutant phenotype.

Table 2.1 KASP marker analyses of parent lines and segregating F_2 plants indicating the pedigree, HCN production characteristics based on the Feigl-Anger Assay, and the CYP79A1 genotype for each sample.

Pedigree	HCN Production	CYP79A1 Genotype
BTx623	Yes	WT / WT
EMS 2447	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	Yes	WT / WT
EMS 2247 x Tx623 F_2	Yes	WT / WT
EMS 2247 x Tx623 F_2	Yes	WT / WT
EMS 2247 x Tx623 F_2	Yes	WT / C493Y
EMS 2247 x Tx623 F_2	Yes	WT / C493Y
EMS 2247 x Tx623 F_2	Yes	WT / C493Y
EMS 2247 x Tx623 F_2	Yes	WT / C493Y
EMS 2247 x Tx623 F_2	Yes	WT / C493Y
EMS 2247 x Tx623 F_2	Yes	WT / C493Y
EMS 2247 x Tx623 F_2	Yes	WT / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y
EMS 2247 x Tx623 F_2	No	C493Y / C493Y

2.3.4 Dhurrin Concentration

Dhurrin concentration of C493Y mutant and wild-type plants was determined by HPLC at the USDA Cropping Systems Laboratory in Lubbock, TX (Table 2.2). Variations in dhurrin concentration were considered significant if the difference is greater than the minimum significant value of 3.24. The parent line BTx623 had the highest overall dhurrin concentration followed by the wild-type plants from the F_2 population. The acyanogenic parent line EMS 2447, the acyanogenic maize check B73, and the

acyanogenic plants from the F_2 family had the lowest dhurrin concentrations and were

not significantly different from each other. Since maize does not produce dhurrin, it can

be assumed that the values given for its mean are a baseline level of absorbance and the

dhurrin negative lines also have negligible to null dhurrin concentrations.

Table 2.2 Mean dhurrin concentration values for parents BTx623 and EMS 2447, wild-type and acyanogenic F_2 plants, and acyanogenic maize. The statistical groupings are based on Tukey's studentized range test.

Pedigree	Generation	Feigl-Anger Assay	Sample Size	Dhurrin ($\mu g/cm^2$)	Statistic Grouping
B Tx623	Parental line	Blue	4	15.03	A
EMS 2447 x Tx623	F_2	Blue	16	11.41	B
EMS 2447	Parental line	White	4	5.40	C
B73 - Maize check	Check	White	8	3.64	C
EMS 2447 x Tx623	F_2	White	16	2.18	C

2.4   Discussion

The main objective of this research project was to identify the genetic mutations that

resulted in the acyanogenic phenotypes exhibited in EMS 2447 and EMS 5085. Using a

workflow developed by Krothapalli et al. (2013), we identified a C493Y mutation in

CYP79A1, the first gene in the dhurrin biosynthetic. We hypothesize that this missense

mutation could disrupt active site of the protein. Blomstedt et al. (2012) identified a

P414L mutation in CYP79A1. To date, C493Y and P414L of CYP79A1 are the only

reported mutations of genes that disrupt production of dhurrin, unlike the mutation

reported by Krothapalli et al. (2013) that produces dhurrin normally but cannot rapidly

break it down due to loss of *dhurrinase2* function.

The study by Krothapalli et al. (2013) was one of the first to describe a gene discovery pipeline based on whole genome resequencing of EMS mutants in a higher crop plant like sorghum. With minor modifications, the workflow used in this study was similar to that described by Krothapalli et al. (2013) and proved to be successful in identification of the causal mutations in EMS 2447 and EMS 5085 as a C493Y mutation in CYP79A1. Given the power of this method for gene discovery, we anticipate that similar methods will be developed in other crop plants and genetics systems.

There are over 2,000 cyanogenic plant species, some of which are economically important crops species where eliminating HCN toxicity could greatly benefit consumers. Cassava (*Manihot esculenta*) produces two cyanogenic glucosides, linamarin and lotaustralin in plant tissues. Although there are no acyanogenic cassava cultivars, traditional breeding has created low HCNp cultivars, and transgenic research was able to reduce cyanogenic glucosides in the tubers by 92% and in the leaves by more than 99% (Jørgensen et al., 2005). These low HCNp cultivars are generally termed "sweet" and often are preferred by end-users because of the greater ease in preparing food from roots of these cultivars (Cardoso et al., 2005). Sorghum breeders have also selected for low HCNp in development of improved forage cultivars. Piper Sudangrass and many of its derivatives were developed by selection for low HCNp in sorghum and are widely grown for pasture, greenchop, hay, and cover crop (Smith et al., 1973). Gorz et al. (1986) demonstrated that the inheritance of the low-HCNp trait was controlled by only a few genes.

Since no dhurrin-free lines have been identified in the standing variation of sorghum (Gorz et al., 1977; Burke et al., 2013; Tuinstra, personal communication, 2013),

it was important to verify that the C493Y mutation in CYP79A1 co-segregated with the acyanogenic phenotype. We demonstrated that the acyanogenic phenotype co-segregated perfectly with homozygous C493Y mutation, and the wild-type plants were either homozygous wild-type or heterozygous. This result could be useful in marker assisted selection for incorporating the C493Y mutation into elite cultivars to improve forage qualities.

Since dhurrin concentrations are highest in young tissues, producers who want to graze sorghum stover after harvest must take into consideration environmental conditions and regrowth that could increase the dhurrin concentration and therefore the HCN toxicity risk to their livestock (Hunt and Taylor, 1976; McBee and Miller, 1980; Sidhu et al. 2011). Results from the current studies suggest that efforts to incorporate the C493Y mutation of CYP79A1 into elite brown midrib forage sorghum varieties with high feed value (Oliver et al. 2005; Sattler et al. 2010) would eliminate the risk of HCN toxicity for animal producers and provide a safer forage source for livestock.

CHAPTER 3. PHENOTYPIC ANALYSIS OF THE IMPACT OF DHURRIN
MUTANTS ON SORGHUM GROWTH AND DEVELOPMENT

3.1   Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop in semiarid

agricultural regions (Borrell et al., 2000a). The crop is produced as food, feed, forage,

and silage in many different parts of the world. One factor that can affect sorghum forage

quality is dhurrin production (Hunt and Taylor, 1975; Busk and Møller, 2002; Etuk et al.,

2012). Dhurrin is a cyanogenic glucoside naturally produced in the plant.

Cyanogenic glucosides are found in many plant species, including ferns,

gynosperms, and angiosperms (Zagrobelny et al., 2004). Dhurrin is the cyanogenic

glucoside particular to sorghum. It is a β-glucoside derived from tyrosine. The three

genes in the anabolic pathway (CYP79A1, CYP71E1, and UGT85B1) transform tyrosine

into dhurrin. It is readily produced by the plant and stored within the leaf cells as a

defensive compound, which classifies it as a phytoanticipin (Zagrobelny et al., 2004;

Møller, 2010). Young sorghum seedlings can produce up to 6% of their dry weight as

dhurrin (Møller, 2010). When sorghum leaf tissue is disrupted or damaged, for example

by chewing of an herbivore, dhurrin is degraded by two β-glucosidases and a

hydroxynitrile lyase, releasing the byproduct HCN. For this reason, dhurrin is shown to

be a defensive compound against herbivores (Hösel et al., 1987; Halkier and Møller,

1989; Morant et al., 2003, Zagrobelny et al., 2004; Krothapalli et al., 2013).

While dhurrin may benefit the sorghum plant by providing a defense against insect herbivory and a potential nitrogen storage compound, it can have detrimental effects to livestock that consume the edible plant tissues. However, aside from the toxicity issue, sorghum has numerous characteristics that make it a desirable forage crop including high dry matter yield, capacity for rapid regrowth, efficient use of water due to its C4 nature, and efficient use of nitrogen in low fertility conditions (Wheeler and Mulcahy, 1989). Reducing or eliminating dhurrin production in sorghum could reduce the risk of toxicity to sorghum consumers.

While there is quantitative genetic diversity for dhurrin production in sorghum, there have been no naturally-occurring, dhurrin-free genotypes identified to date (Hogg and Ahlgren, 1943; Gorz et al., 1977; Blomstedt et al., 2012; Burke et al., 2013). Blomstedt et al. (2012) demonstrated that EMS mutagenesis could be used to develop dhurrin-free mutants. They identified a mutant line with a P414L mutation in CYP79A1 that caused complete disruption of dhurrin biosynthesis. The only differences they noted between the acyanogenic mutant and the control genotype was slower growth in the seedling stage that was not exhibited in the adult stage. Krothapalli et al. (2013) used a similar approach to produce an EMS mutant genotype defective in HCN release. This mutant produced dhurrin but was not able to convert dhurrin into HCN. It was determined that the causal mutation was in dhurrinase-2. These studies demonstrate that it is possible to produce acyanogenic sorghum mutants using EMS chemical treatments. If these mutations can be incorporated into elite varieties without negatively affecting yield or other forage qualities, this could lead to sorghum cultivars with reduced dhurrin concentration and risk of HCN toxicity.

Stay-green is one of the most important drought adaptation traits in sorghum. The expression of stay-green has been associated with increased yield under drought conditions through altered nitrogen status of the plant and increased duration of photosynthetically active leaves (Borrell and Hammer, 2000; van Oosterom et al., 2010a). The stay-green trait is defined as extended foliar greenness during the grain filling period, or the retention of green leaf area under post-anthesis drought stress (Borrell et al., 2001). By retaining photosynthetic capabilities, drought-stressed plants with stay-green have less yield loss compared to drought-stressed plants that senesce at normal rates (Thomas and Smart, 1993; Borrell et al., 2000b; Borrell et al., 2001). Increased stay-green under drought stress has been shown to reduce susceptibility to stalk rotting pathogens and lodging when compared to lines that senesce normally (Thomas and Smart, 1993; Borrell et al., 2000b).

Several quantitative trait loci associated with drought tolerance and stay-green have been identified in sorghum (Tuinstra et al., 1997; Xu et al., 2000; Kebede et al., 2001; Sanchez et al., 2002; Harris et al., 2007). These QTL were named *Stg1*, *Stg2*, *Stg3* and *Stg4* and account for over 40% of the phenotypic variation in leaf senescence and chlorophyll content under post-flowering drought stress.

Borrell et al. (2000a and 2000b) showed that stay-green increased yield under drought conditions. The stay-green trait has also been shown to enhance feed quality of dual-purpose sorghums, especially in the semi-arid tropics (Borrell et al., 2000b; Zerbini and Thomas, 2003). Functional stay-green during grain filling contributes to increased accumulation of soluble sugars, which reduces the plant's dependence on stored assimilates to fill the grain. This increase in stem sugars results in improved quality of the

stover for use in animal feed. If this improved nutrition could be combined with lower lignin content brown mid-rib type sorghums, there could be potential for even further improvement in sorghums used as livestock feed (Sattler et al., 2010).

There are two sources of nitrogen for the plant to use in producing seed. One source is the soil available nitrogen absorbed through the roots. The other source is nitrogen stored in and remobilized from the other plant tissues. Both of these sources can affect the consistency of stay-green expression in sorghum (Borrell and Hammer 2000; van Oosterom et al., 2010b). However, remobilized nitrogen in the plant is the key source when plants are grown in nitrogen-limited or drought-prone environments (Borrell et al., 2001). When plants begin to senesce, they cease to produce amino acids and instead degrade existing proteins and remobilize amino acids out of the leaf (Borrell and Hammer, 2000). While genetic components can increase expression of stay-green, it can also be attributed to the balance between N demand for grain fill and the available N supply (Borrell and Hammer, 2000; Borrell et al., 2001).

Burke et al. (2013) showed that dhurrin content in sorghum seedlings was correlated with increased stay-green capacity at maturity. Since dhurrin is believed to be a nitrogen storage compound, the relationship between dhurrin and stay-green may reflect differences in the nitrogen status of the plant. Therefore, it would be reasonable to speculate that increased dhurrin content could mean an improved nitrogen status that leads to increased stay-green later in the life of the plant. However, dhurrin content of sorghum is highest during the seedling stage and decreases in the plant as it matures (McBee and Miller, 1980; Busk and Møller, 2002), although drought stress can also increase dhurrin concentration (O'Donnell et al., 2013). Thus, the causal relationships

between dhurrin content during the seedling stage and stay-green at physiological maturity are yet to be determined.

The objectives of this research project were to determine the effects of dhurrin loss on sorghum growth and development, as well as gain a better understanding of the relationship between dhurrin content and stay-green in sorghum. EMS-induced genetic mutants were used to test the hypothesis that lack of dhurrin will not affect growth and development. Field studies were conducted under nearly ideal environmental conditions and gave rise to questions about the dhurrin mutants and their expression of stay-green under drought conditions. Genetic mutants were used to test a second hypothesis that lack of dhurrin production or disrupted dhurrin catabolism negatively impact expression of stay-green under drought conditions in greenhouse trials.

3.2   Materials and Methods

3.2.1   Objective 1: Characterizing Growth and Development of Dhurrin Mutants

Four inbred genotypes contrasting for *Cyp79A1-2* were planted, including EMS 2447 that was homozygous for *Cyp79A1-2* and does not produce dhurrin, as well as three normal genotypes including BTx623, the reference genome for *Sorghum bicolor*; Tx2737, a common R-line; and 915B, a brown-midrib breeding line. F_2 families segregating for *Cyp79A1-2* were created from crosses of EMS 2447 with BTx623, Tx2737, and 915B. Field plots were planted at Ag Alumni in Romney, IN (May 30, 2013) and at Purdue University's Agronomy Center for Research and Education (ACRE) in West Lafayette, IN (June 5, 2013). Field management and chemical applications for the Romney location are as follows. The field was tilled in the fall. In the spring one month prior to planting,

28% urea-ammonium nitrate (UAN) was applied at a rate of 180 kg ha^{-1} along with Bicep II Magnum [atrazine and S-metolachlor (Syngenta Crop Protection, Inc.)] at 3.8 liters ha^{-1} and Princep 4L [simazine (Syngenta Crop Protection, Inc.)] at 4.7 liters ha^{-1}. After emergence, no further chemicals were used and weeds were controlled by hoeing. Field management and chemical applications for the ACRE location are as follows. In the fall prior to planting, 340 kg ha^{-1} of potash as KCl was applied after the previous crop (soybean) had been removed. Three weeks later the field was chisel plowed. In the spring, one month prior to planting, 170 kg ha^{-1} of anhydrous ammonia was applied. Weeds were controlled by treating plots with Bicep II Magnum [atrazine and S-metolachlor (Syngenta Crop Protection, Inc.)] at 3.8 liters ha^{-1} and Roundup [glyphosate (Monsanto Company)] with ammonium sulfate (to treat hard water) at 1.75 liters ha^{-1} after planting, then by hoeing later in the growing season.

The four parent lines were planted in a randomized complete block design with four blocks. Each single row plot was approximately four meters in length. The F$_2$ families were planted in a randomized complete block design in plots approximately four meters in length with a space of 0.76 meters between each plot and 16 blocks. Two rows of BTx623 were planted on either side of the experimental plots for the entire length of the experiment for border. Since both wild-type and mutant-type plants were sampled from each individual plot and different plants were sampled throughout the season, it is considered a split-plot in time design.

In the segregating F$_2$ families, 24 plants from each plot were sampled using the Feigl-Anger assay (Feigl and Anger, 1966; Krothapalli et al., 2013) to identify mutant and wild-type plants in each row. A 2.5 cm section of the youngest leaf of a sorghum

seedling was sampled using scissors and gently placed into a well on a 96-well polystyrene flat-bottomed microtiter plate using forceps and frozen in a -80 ºC freezer for a minimum of two hours. The sample plates were removed from the freezer and exposed to Feigl-Anger Assay sheets prepared as previously described (Krothapalli et al., 2013). A Feigl-Anger Assay sheet was placed on top of the plate wells and creased on all edges of the plate to ensure a snug fit between the plate and the lid and placed in an incubator at 35 ºC to thaw the tissue. The plates were incubated for 10 to 15 minutes until blue spots were visible on the Whatman paper. The plates were removed from the incubator and the reaction for each individual plate well was recorded. Wells that contained wild-type tissue released HCN that reacted with the paper, producing a blue spot. These wells were phenotyped as "blue". Tissues that lacked HCN production failed to react with the paper and no color change was observed, thus these wells were phenotyped as "white", the original color of the treated Whatman paper. Plants that were characterized as white were re-sampled to verify their phenotype and individually labeled in the field for later identification throughout the growing season.

Individual mutant and wild-type F_2 plants from each plot were phenotyped throughout the growing season to compare growth characteristics between wild-type and dhurrin negative plants within each F_2 family. Plant height, chlorophyll content index, leaf number, and biomass were sampled at 3, 6, and 17 weeks (harvest) after planting on one wild type and one dhurrin negative plant in each plot. Chlorophyll measurements were also taken midseason at Week 12 during anthesis. Plant height was measured from the soil line to the top of the curve of the uppermost fully expanded leaf or top of the head (Armbrust and Bilbro, 1993). The height stick was marked off in inches and

measurements were later converted to centimeters. Chlorophyll measurements were taken

using an Opti-Sciences CCM 200 Plus chlorophyll content meter (Opti-Sciences, Inc.).

The CCM 200 Plus provides a chlorophyll content index (CCI), a ratio of absorbance of

two wavelengths, 931 nm and 653 nm. The ratio is calculated by the following equation:

$$CCI = \frac{\%\ transmittance\ at\ 931\ nm}{\%\ transmittance\ at\ 653\ nm}$$

Leaf number was determined by the number of fully expanded leaves at the time of

sampling. Fully expanded leaves had no overlap at the leaf collar. Biomass samples were

harvested by cutting the plant at the soil line and keeping all aboveground plant tissues.

The samples from the 3- and 6-week sampling times were placed in labeled paper bags

and dried at 60 °C for three days. At the harvest sampling time, the head was cut and

placed in a mesh bag while the remaining biomass was placed in a separate burlap bag.

The biomass was dried at 60 °C for three days while the heads were dried at 35 °C for

three days. After drying, the heads were threshed for grain yield and the panicle was

added to the aboveground biomass measurement. Harvest index was determined using the

following calculation:

$$harvest\ index = \left(\frac{grain\ yield}{grain\ yield + biomass}\right)$$

3.2.2 Objective 2: Dhurrin Content and Stay-Green

A greenhouse experiment was conducted to test wild-type sorghum genotypes and

dhurrin mutants for differences in stay-green under optimal and water-limited conditions.

BTx623, the reference genome of sorghum and the genotype mutagenized for these

studies, was used as the control genotype with normal dhurrin production. A homozygous genotype with the C493Y mutation in CYP79A1 (*Cyp79A1-2*) was selected from a backcross to Tx623 and seed was produced by self-pollination. This genotype does not produce dhurrin and exhibits normal growth. A homozygous plant with a mutation in *dhurrinase2* (*dhr2-1*) was identified (Krothapalli et al., 2013) and seed was produced by self-pollination. This line produces dhurrin normally but is not able to break it down and release HCN due to a Tryptophan 194 Stop mutation in Dhr2. BTx623, *Cyp79A1-2*, and *dhr2-1* were evaluated under drought stress and well-watered conditions to compare differences in expression of stay-green among lines with different dhurrin production and degradation characteristics.

Seedlings of BTx623, *Cyp79A1-2*, *dhr2-1*, and an acyanogenic maize check (B73) were planted in a washed sand bench in the greenhouse. Plants were managed on a 14 hour day length, watered daily, and kept between 32 ºC during the day and 24 ºC at night. After verifying the phenotypes of these genotypes using a Feigl-Anger assay, the seedlings were sampled for determination of dhurrin concentration through HPLC. A Harris Uni-Core (Shunderson Communications, Inc.) 6.00 mm punch and Harris self-healing cutting mat were used to take leaf tissue samples. Five punches from the second leaf of each plant were sampled midway between the leaf base and tip. Samples were placed into microcentrifuge tubes. The samples were stored on ice until returned to the lab where they were stored at -20°C. Tissue samples for HPLC were shipped on dry ice to John Burke at the USDA Cropping Systems Laboratory in Lubbock, TX for analysis of dhurrin concentration using the HPLC protocol described in Burke et al. (2013).

Thirty 30 x 35 cm plastic pots (volume $\approx$ 30270 cm^3) were filled with a soil

mixture containing a 1:1:1:1 ratio of field soil, sand, Metro-Mix 510 growing medium

(Sun-Gro Horticulture Canada Ltd.) and Turface Athletics MVP (Profile Products LLC)

soil conditioner. The pots were watered to field capacity and seeds from BTx623,

Cyp79A1-2, and *dhr2-1* were planted in separate hills in all thirty pots. Plants were

managed on a 14 hour day length with greenhouse temperature between 24 ºC at night

and 32 ºC during the day. Photosynthetically active radiation (PAR) was measured with a

LI-250A light meter (LI-COR, Inc.) on a sunny day and averaged 650 µmol s^{-1} m^{-1}.

Figure 3.1 Plants of BTx623, EMS 932 (*dhr2-1*), and an F$_3$ selection of EMS 5085 x
Tx623 (*Cyp79A1-2*) planted together in the same pots for the stay-green and drought
experiment, Fall 2013.

Once the emerged seedlings were large enough to sample (approximately one

week), the stands were thinned to one plant per line in each pot and tissue samples were

collected for the Feigl-Anger assay (Section 3.2.3). The wild-type BTx623 produces

dhurrin and generates a blue spot in the Feigl-Anger assay. The *dhr2-1* mutant line

produces dhurrin normally but lacks production of *dhurrinase2* and cannot break down

dhurrin normally. This line does not initially produce a blue spot in the Feigl-Anger

assay, but if the assay is allowed to run overnight, *dhr2-1* mutant plants will produce a

blue spot on the test paper by the morning. The *Cyp79A1-2* mutants fail to produce

dhurrin. Lack of dhurrin production results in no blue spot on the HCN test paper. The

plants in all but one pot met the expected Feigl-Anger assay results. The exception was

removed from the study. All pots were watered on alternate days to field capacity with

reverse osmosis water until anthesis. Pots were fertilized five weeks after planting with

Osmocote Outdoor and Indoor Smart-Release Plant Food (The Scotts Company LLC) at

a rate of 160 kg ha^{-1} N, 47 kg ha^{-1} P, and 93 kg ha^{-1} K, then again at six weeks after

planting with Miracle-Gro All Purpose Plant Food (The Scotts Company LLC) at a rate

of 75 kg ha^{-1} N, 33 kg ha^{-1} P, and 47 kg ha^{-1} K.

   To compare differences in stay-green capacity between the three lines under well-

watered and drought-stressed conditions, chlorophyll meter readings were taken weekly

beginning three weeks after planting until physiological maturity. Chlorophyll

measurements were taken using an Opti-Sciences CCM 200 Plus portable chlorophyll

meter (Opti-Sciences, Inc.), the same instrument as used for the field measurements.

   When 90% of the plants had reached anthesis, watering was reduced for half of

the pots to induce drought stress. Drought treatment pots were kept below 25% soil

volumetric water content. Soil water properties were measured using a HydroSense II

handheld soil water sensor (Campbell Scientific, Inc.). This device uses soil dielectric

permittivity to measure soil volumetric water content in percent. The volumetric water

content was used to verify differences in soil water content between the drought and well-

watered treatments. Relative water content (RWC) was measured on the plants in each

pot to quantify differences in drought stress between the well-watered and drought

treatments. Tissue samples from the uppermost leaf of each plant were taken using a

Harris Uni-Core 6.00 mm punch and Harris self-healing cutting mat (Shunderson

Communications, Inc.). Each sample was weighed immediately to get a fresh weight

(FW) value in milligrams, then placed in a 1.5 ml microcentrifuge tube with one ml of

distilled water. The samples were allowed to hydrate for 48 hours, then samples were

blotted on a Kimwipe and reweighed to get their turgid weight (TW). Samples were then

placed into clean, dry tubes and dried in an incubator at 50ºC for 48 hours. Samples were

then weighed for a final dry weight (DW). The formula for calculating RWC is as

follows:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \; X \; 100$$

3.2.3 Statistical Analyses

The statistical analyses used for field and greenhouse experiments were performed using

Statistical Analysis Software (SAS Institute Inc.). The SAS code used for analysis is

provided in Appendix A. The growth and development characteristics of the parent lines

in the field experiment were analyzed as a randomized complete block design using

Analysis of Variance (ANOVA). The parent lines had equal samples sizes at all sampling

times and were compared to each other using a t-test and a least significant difference

(LSD) value with an alpha value of 0.05. The LSD value for each sampling time and location is provided in the following tables of data.

Individual and combined analyses of the F_2 population were conducted due to the inherent differences among the parent lines and the presence of Pedigree x C493Y interaction for some traits. Within each population and location, the means between the wild-type and dhurrin negative plants were compared to each other to see if lack of dhurrin production significantly affected growth and development. Combined analyses were also conducted over Pedigrees and Locations since few signification interaction effects were detected. Due to germination issues, there were not always enough C493Y plants in each plot for all sampling times, which led to unequal sample sizes and therefore made it impractical to use the ANOVA procedure. The GLM procedure was used to calculate the analysis of variance table for the unbalanced data and Tukey's multiple comparisons P-value for the least square means (lsmeans) for each phenotypic trait to determine if the differences between the means of the wild-type and dhurrin negative plants were statistically significant.

For the drought experiment, a repeated measures experiment was used since all CCI measurements were taken on the same plants every sampling time. The CCI values for the three genotypes were compared using the ANOVA procedure to calculate the analysis of variance table and perform the student's t-test of least significant differences to determine if the means between the three lines were statistically significant with an alpha value of 0.05. This procedure was used for the first seven sampling times. After the drought treatment was applied, the ANOVA procedure was still used, but the Waller-Duncan k-ratio t-test multiple comparison procedure in the means statement was used to

compare the chlorophyll means between the three lines and two watering regiments instead of the student's t-test. Each line and water treatment combination, a total of six, were given an individual code letter to use in the analysis. For the volumetric water content between the well-watered and drought treatments, the student's t-test of least significant differences was used to determine statistical significance.

3.3   Results

3.3.1   Growth and Development Characteristics of Parent Lines

The growth and development characteristics in the parent lines are presented in the following tables. Chlorophyll content index (CCI) of the parent lines is presented in Table 3.1. At the Week 3 sampling time at ACRE, BTx623 and EMS 2447 had the highest CCI, however EMS 2447 overlapped in the significance group with both 915B and Tx2737. These were the lowest averages throughout the season. Perhaps this was due to slightly different fertility treatments between the two locations or different soil types which accounted for different nutrient availability early in the season. At Week 6, Tx2737 and EMS 2447 had the highest CCI. EMS 2447 also overlapped in the statistical group with 915B and BTx623. At Week 12 and harvest, Tx2737 was significantly higher than the other three parents. At Romney, the parent lines demonstrated no significant differences in CCI at the Week 3 sampling time. At Week 6, EMS 2447 and Tx2737 had the highest CCI and were significantly different from BTx623, but 915B overlapped both groups. At Week 12, Tx2737 had the highest CCI value but no significant differences were detected among lines. At harvest, Tx2737 again had the highest mean and was

significantly different from 915B and BTx623, and EMS 2447 overlapped both groups.

The LSD values increased throughout the season, likely due to the larger variation in

plant height and biomass accumulation in the parent lines that could subsequently lead to

reduced chlorophyll concentration.

Table 3.1 Chlorophyll content index (CCI) averages of the four parent lines at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni in Romney, IN. The means were compared using a *t*-test and analyzed by separate location at each sampling time. Means with a different statistical grouping letter (Group) are significantly different from each other based on a least significant difference (LSD) value.

Parent Lines	Chlorophyll Content Index				
	Week 3		Week 6		
ACRE	**LSD: 5.32**	**Group**	**LSD: 8.26**	**Group**	
915B	18.9	B	39.4	B	
BTx623	27.8	A	37.4	B	
Tx2737	20.8	B	50.4	A	
EMS 2447	23.9	AB	42.3	AB	
ROMNEY	**LSD: 10.91**	**Group**	**LSD: 12.60**	**Group**	
915B	31.2	A	47.5	AB	
BTx623	34.8	A	36.8	B	
Tx2737	28.5	A	50.4	A	
EMS 2447	27.8	A	52.2	A	

Parent Lines	Chlorophyll Content Index				
	Week 12		Harvest		
ACRE	**LSD: 9.78**	**Group**	**LSD: 18.81**	**Group**	
915B	50.2	B	39.5	B	
BTx623	49.4	B	32.1	B	
Tx2737	79.7	A	62.2	A	
EMS 2447	46.7	B	20.8	B	
ROMNEY	**LSD: 22.12**	**Group**	**LSD: 17.65**	**Group**	
915B	47.2	A	38.7	B	
BTx623	46.7	A	31.5	B	
Tx2737	53.6	A	56.9	A	
EMS 2447	39.3	A	41.2	AB	

At ACRE, 915B and BTx623 were the tallest at Week 3, EMS 2447 was the shortest, and Tx2737 was in the middle and overlapped both groups (Table 3.2). At Week 6, BTx623 was the tallest. 915B and TX2737 overlapped with both the highest and lowest groups. EMS 2447 remained the shortest. At harvest, the parent lines were significantly different from each other with 915B being the tallest at 184.8 cm, followed by BTx623 at 146.3 cm, then Tx2737 at 116.8 cm, and EMS 2447 was the shortest at 97.8 cm. At Romney, no differences in plant height were detected at Week 3. At Week 6, there was more variation in plant height but the means overlapped for all lines. 915B was the tallest followed by BTx623, then Tx2737, and finally EMS 2447 was the shortest. At harvest, 915B was much taller than the other parent lines at 191.5 cm followed by BTx623 at 168.8 cm. Tx2737 was similar in height to EMS 2447.

Table 3.2 Height means of the four parent lines at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni in Romney, IN. The means were compared using a *t*-test and analyzed by separate location at each sampling time. Means with a different statistical grouping letter (Group) are significantly different from each other based on a least significant difference (LSD) value.

Height (cm)	Week 3		Week 6		Harvest	
ACRE	LSD: 3.44	Group	LSD: 13.35	Group	LSD: 17.93	Group
915B	31.8	A	76.2	AB	184.8	A
BTx623	31.1	A	81.9	A	146.3	B
Tx2737	29.8	AB	72.4	AB	116.8	C
EMS 2447	27.3	B	63.5	B	97.8	D
ROMNEY	LSD: 7.74	Group	LSD: 8.42	Group	LSD: 13.20	Group
915B	28.6	A	85.1	A	191.5	A
BTx623	27.3	A	80.6	AB	168.8	B
Tx2737	23.5	A	73.0	BC	123.0	C
EMS 2447	24.8	A	69.9	C	111.3	C

Parent lines showed more variability in leaf number at ACRE than at Romney

(Table 3.3). At ACRE, plants averaged 4 to 5 leaves per plant at Week 3, while at Week 6

the averages ranged from 7 to 8 leaves per plant. BTx623 and Tx2737 averaged slightly

higher leaf number than 915B and EMS 2447 at both sampling dates. At Romney, there

were no statistical differences in leaf number among the parent lines at either sampling

time. Leaf number was not taken at the harvest sampling time since many of the lower

leaves had senesced or fallen off, which made it difficult to get an accurate leaf count.

Table 3.3 Leaf number means for the four parent lines at Purdue University Agronomy
Center for Research and Education (ACRE) and Ag Alumni in Romney, IN. The means
were compared using a *t*-test and analyzed by separate location at each sampling time.
Means with a different statistical grouping letter (Group) are significantly different from
each other based on a least significant difference (LSD) value.

Leaf Number	Week 3		Week 6	
ACRE	LSD: 0.80	Group	LSD: 0.94	Group
915B	4.8	B	7.3	B
BTx623	5.8	A	8.0	AB
Tx2737	5.5	AB	8.5	A
EMS 2447	4.8	B	7.8	AB
ROMNEY	LSD: 0.86	Group	LSD: 1.18	Group
915B	4.8	A	7.5	A
BTx623	4.5	A	8.0	A
Tx2737	4.5	A	8.5	A
EMS 2447	4.5	A	8.5	A

Further evidence of the variation in growth and development of the parents is

shown in the biomass dry weights (Table 3.4). Plants at Week 3 showed no significant

differences in biomass at ACRE. At Week 6, 915B had the highest biomass, EMS 2447

had the lowest biomass, and BTx623 and Tx2737 overlapped both groups in the middle.

However, by harvest, there were three distinct biomass groups. 915B, which was also the

tallest line, had the highest biomass at nearly 200 gm. BTx623 produced significantly

lower biomass than 915B at 145.9 gm. At the low end, Tx2737 and EMS 2447 were in

the bottom statistical group with 95.0 and 71.6 gm, respectively. At Romney, no

statistically significant differences in biomass were observed at Weeks 3 and 6. However,

at harvest, 915B was the tallest and had the greatest biomass at 258.5 gm, nearly double

the biomass of the other parent lines that fell into a separate group with the mutant parent

EMS 2447 having the lowest biomass.

Table 3.4 Biomass dry weight means for the four parent lines at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni in Romney, IN. The means were compared using a *t*-test and analyzed by separate location at each sampling time. Means with a different statistical grouping letter (Group) are significantly different from each other based on a least significant difference (LSD) value.

Biomass (gm)	Week 3		Week 6		Harvest	
ACRE	LSD: 1.04	Group	LSD: 20.24	Group	LSD: 40.21	Group
915B	2.1	A	47.0	A	199.7	A
BTx623	2.4	A	38.2	AB	145.9	B
Tx2737	2.4	A	38.3	AB	95.0	C
EMS 2447	1.8	A	22.3	B	71.6	C
ROMNEY	LSD: 1.31	Group	LSD: 17.99	Group	LSD: 68.90	Group
915B	1.5	A	32.2	A	258.5	A
BTx623	1.5	A	30.5	A	132.9	B
Tx2737	1.0	A	21.3	A	83.5	B
EMS 2447	1.2	A	24.7	A	78.3	B

Grain yield for the parent lines varied but the mutant parent EMS 2447 had the lowest yield of all the lines at both locations (Table 3.5). At ACRE, BTx623 had the highest grain yield but was not statistically different from 915B, which also overlapped with Tx2737. EMS 2447 was significantly lower than all lines. At Romney, there were two statistical groups. 915B and BTx623 were the highest and TX2737 was grouped with EMS 2447 in the lowest yielding group

The lower grain yield of EMS 2447, along with shorter height and decreased biomass production are likely due to the effects of the multiple EMS mutations within the background. For this reason, it was important to use F_2 populations for the growth and development experiment to reduce these effects.

Table 3.5 Grain yield means for the four parent lines at harvest at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni in Romney, IN. The means were compared using a *t*-test and analyzed by separate location. Means with a different statistical grouping letter (Group) are significantly different from each other based on a least significant difference (LSD) value.

Grain Yield (gm)					
ACRE	LSD: 24.22	Group	ROMNEY	LSD: 27.58	Group
915B	74.8	AB	915B	83.0	A
BTx623	91.7	A	BTx623	77.9	A
Tx2737	65.0	B	Tx2737	47.4	B
EMS 2447	31.1	C	EMS 2447	32.7	B

Harvest index varied between the parent lines at ACRE but the differences in harvest index at Romney were not significant (Table 3.6). At ACRE, Tx2737 and BTx623 had the highest harvest index values. 915B grouped with EMS 2447 in the

lowest harvest index group. The harvest index of 915B was low because it produced the most biomass. The harvest index of EMS 2447 was low because it had low biomass and low grain yield.

Table 3.6 Harvest index means for the four parent lines at harvest at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni in Romney, IN. Harvest index was calculated as grain yield divided by total biomass and grain yield. The means were compared using a *t*-test and analyzed by separate location. Means with a different statistical grouping letter (Group) are significantly different from each other based on a least significant difference (LSD) value.

Harvest Index					
ACRE	LSD: 0.06	Group	ROMNEY	LSD: 0.15	Group
915B	0.28	B	915B	0.24	A
BTx623	0.38	A	BTx623	0.36	A
Tx2737	0.41	A	Tx2737	0.39	A
EMS 2447	0.32	B	EMS 2447	0.34	A

3.3.2   Growth and Development Characteristics of F_2 Families

As shown above, the parent lines varied in growth and development characteristics and these differences in phenology confounded efforts to directly compare the dhurrin-free mutant phenotypes. Sets of F_2 families were created to evaluate if differences in phenology and growth were associated with the C493Y mutation segregating in each family. ANOVA of the phenotypes in each family and sampling time are shown in Table 3.7. Analyses of chlorophyll content showed that Location was significant at each sampling time and Pedigree was significant at Week 12 and harvest. However, C493Y and the Pedigree x C493Y interaction were not significant at any time point. Analyses of plant height showed that Location and Pedigree were significant at Week 6 and harvest.

The C493Y main effect was significant for plant height at each sampling time but the Pedigree x C493Y interaction was not significant. Analyses of leaf number showed that Location was not significant. The C493Y main effect was significant at Week 3 and Pedigree was significant at Week 6. Analyses of biomass yield showed no significant effects at Week 3; however, Location and C493Y was significant at Week 6. Location, Pedigree, and C493Y were significant at harvest.

Table 3.7 Analysis of Variance (ANOVA) for chlorophyll content index, plant height, leaf number, and biomass sampled at multiple times throughout the growing season. The mean square values and significance are provided for each source of variation in the statistical model. Sources of variation were considered to be significant if the P-value was less than 0.05. Significance levels are indicated by: *** for 0.001, ** for 0.01, and * for 0.05.

Source	Chlorophyll Content Index							
	Week 3		Week 6		Week 12		Harvest	
	Mean square		Mean square		Mean square		Mean square	
Location	3597.7	***	2990.7	***	44.8	NS	2065.9	***
Pedigree	31.4	NS	173.1	NS	2723.8	***	2663.3	***
C493Y	36.4	NS	15.4	NS	268.4	NS	242.7	NS
Pedigree x C493Y	2.6	NS	152.3	NS	111.3	NS	170.6	NS

Source	Plant Height							
	Week 3		Week 6		Harvest			
	Mean square		Mean square		Mean square			
Location	1.2	NS	2594.4	***	2630.1	**		
Pedigree	47.5	NS	674.5	***	65889.8	***		
C493Y	194.4	**	1562.4	***	1575.8	*		
Pedigree x C493Y	14.1	NS	95.4	NS	144.7	NS		

Source	Leaf Number							
	Week 3		Week 6					
	Mean square		Mean square					
Location	1.28	NS	0	NS				
Pedigree	0.54	NS	18.5	***				
C493Y	2.50	*	2.22	NS				
Pedigree x C493Y	0.53	NS	1.36	NS				

Source	Biomass							
	Week 3		Week 6		Harvest			
	Mean square		Mean square		Mean square			
Location	0.28	NS	13176	***	12630	*		
Pedigree	0.53	NS	348	NS	168475	***		
C493Y	3.34	NS	2880	*	13173	*		
Pedigree x C493Y	0.89	NS	470	NS	1444	NS		

At ACRE, the only family with a significant difference in CCI between the mutant-type and wild-type was 2447 x 915B at the Week 3 sampling time (Table 3.8).

The C493Y mutant exhibited the higher CCI value. At Romney, there were no significant differences observed at Week 3. At Week 6, 2447 x Tx623 was significantly different with the C493Y mutant exhibiting the higher CCI value. Tx2737 x 2447-1 was also significantly different but the wild-type exhibited the higher CCI value. No significant differences were observed in 2447 x 915B or Tx2737 x 2447-2. No significant differences were observed in any family at Week 12 and harvest at either location. The combined analysis showed no significant differences between the wild-type (WT) and C493Y plants for CCI at any sampling time.

Table 3.8 Chlorophyll content index (CCI) for the F_2 families measured at four sampling times at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni (Romney). The least squares means of the wild-type (WT) and acyanogenic mutant (C493Y) F_2 plants in each family were compared at each location and sampling time as well as averaged over locations and families. Differences (DIF) are considered

significant if the P-value was less than 0.05. Significant (S) differences are highlighted in red and non-significant (NS) are not highlighted.

Chlorophyll Content Index	Week 3			Week 6		
	WT	C493Y	DIF	WT	C493Y	DIF
ACRE						
2447 x 915B	23.2	27.9	S	40.9	41.7	NS
2447 x Tx623	23.9	24.1	NS	38.2	37.9	NS
Tx2737 x 2447-1	26.0	26.2	NS	42.4	43.6	NS
Tx2737 x 2447-2	24.5	24.4	NS	38.5	43.2	NS
ROMNEY						
2447 x 915B	34.3	31.9	NS	45.4	46.0	NS
2447 x Tx623	34.2	35.0	NS	41.6	50.8	S
Tx2737 x 2447-1	29.4	32.4	NS	52.3	45.6	S
Tx2737 x 2447-2	31.2	32.0	NS	52.9	45.7	NS
AVERAGE	28.3	29.1	NS	44.0	44.5	NS

Chlorophyll Content Index	Week 12			HARVEST		
	WT	C493Y	DIF	WT	C493Y	DIF
ACRE						
2447 x 915B	50.0	46.8	NS	36.0	34.4	NS
2447 x Tx623	56.3	47.3	NS	28.9	29.9	NS
Tx2737 x 2447-1	58.4	56.0	NS	40.2	42.5	NS
Tx2737 x 2447-2	60.9	61.3	NS	40.9	39.0	NS
ROMNEY						
2447 x 915B	49.5	50.8	NS	38.2	35.0	NS
2447 x Tx623	46.9	42.5	NS	37.6	31.6	NS
Tx2737 x 2447-1	64.1	66.4	NS	50.4	53.4	NS
Tx2737 x 2447-2	62.2	56.4	NS	49.8	41.3	NS
AVERAGE	56.0	53.8	NS	40.2	38.1	NS

At ACRE, there were statistically significant differences for the mutation in the

2447 x Tx623 and Tx2737 x 2447-2 families at Week 3 with the wild-type plants taller in

both cases (Table 3.9). At Week 6, Tx2737 x 2447-1 was the only family with significant

differences between groups, also in favor of the wild-type. At harvest, Tx2737 x 2447-1

again exhibited significant differences between groups with the wild-type plant being
taller that the C493Y mutants. At Romney, there were no significant differences in plant
height in any family at Week 3 or Week 6 but statistically significant differences were
observed in 2447 x Tx623 at harvest. The combined analysis indicated that the wild-type
plants were slightly taller than the C493Y mutants at Week 3, Week 6, and at harvest.

Table 3.9 Plant heights for the F_2 families measured at three sampling times at Purdue
University Agronomy Center for Research and Education (ACRE) and Ag Alumni
(Romney). The least squares means of the wild-type (WT) and acyanogenic mutant
(C493Y) F_2 plants in each family were compared at each location and sampling time as
well as averaged over locations and families. Differences (DIF) are considered significant
if the P-value was less than 0.05. Significant (S) differences are highlighted in red and
non-significant (NS) are not highlighted.

Height (cm)	Week 3			Week 6			Harvest		
	WT	C493Y	DIF	WT	C493Y	DIF	WT	C493Y	DIF
ACRE									
2447 x 915B	30.1	30.3	NS	86.2	78.0	NS	190.4	181.2	NS
2447 x Tx623	27.5	24.7	S	81.5	77.6	NS	124.1	118.9	NS
Tx2737 x 2447-1	30.0	28.7	NS	86.0	75.7	S	128.1	121.3	NS
Tx2737 x 2447-2	29.5	26.0	S	86.2	82.6	NS	131.1	122.1	S
ROMNEY									
2447 x 915B	28.7	27.8	NS	88.3	80.4	NS	202.8	198.6	NS
2447 x Tx623	31.9	29.5	NS	77.8	72.4	NS	127.4	112.4	S
Tx2737 x 2447-1	26.7	24.5	NS	71.0	67.4	NS	127.0	125.1	NS
Tx2737 x 2447-2	28.4	27.6	NS	71.4	70.2	NS	136.0	136.2	NS
AVERAGE	29.2	27.4	S	81.0	76.0	S	145.8	140.3	S

By the time the plants had reached maturity, many of the lower leaves had
senesced, making it difficult to make an accurate count of leaves present. At ACRE,
TX2737 x 2447-2 showed significant differences in leaf number at Week 3 and Week 6
(Table 3.10). No other families had any significant differences in leaf number at either

sampling time. At Romney, Tx2737 x 2447-1 exhibited a significant difference in leaf number at Week 3, and Tx2737 x 2447-2 was significantly different at Week 6. Neither 2447 x 915B nor 2447 x Tx623 showed any significant differences at either sampling time. The combined analysis indicated that the wild-type plants had a slightly higher leaf number than the C493Y mutants at Week 3, but these differences were not significant at Week 6.

Table 3.10 Leaf numbers for the F_2 families measured at two sampling times at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni (Romney). The least squares means of the wild-type (WT) and acyanogenic mutant (C493Y) F_2 plants in each family were compared at each location and sampling time as well as averaged over locations and families. Differences (DIF) are considered significant if the P-value was less than 0.05. Significant (S) differences are highlighted in red and non-significant (NS) are not highlighted.

Leaf Number	Week 3	Week 6

	WT	C493Y	DIF	WT	C493Y	DIF
ACRE						
2447 x 915B	5	5	NS	8	8	NS
2447 x Tx623	5	5	NS	8	8	NS
Tx2737 x 2447-1	6	5	NS	9	9	NS
Tx2737 x 2447-2	6	5	S	9	9	S
ROMNEY						
2447 x 915B	5	5	NS	8	8	NS
2447 x Tx623	6	6	NS	8	9	NS
Tx2737 x 2447-1	6	5	S	9	9	NS
Tx2737 x 2447-2	6	6	NS	9	9	S
AVERAGE	6	5	S	9	8	NS

Significant differences in biomass were not observed for any family at either location at Week 3 (Table 3.11). At ACRE, 2447 x 915B showed significant differences in biomass at Week 6. The wild-type plants had a higher biomass than C493Y mutants. All other families were similar, though the differences were not significant. At harvest, 2447 x Tx623 and Tx2737 x 2447-1 were not significantly different; however, Tx2737 x 2447-2 and 2447 x 915B exhibited significant differences between the pairs. The wild-type plants had a higher biomass than C493Y mutants by over 60 gm in the case of 2447 x 915B. At Romney, Tx2737 x 2447-2 exhibited a significant difference in leaf number at Week 6; however, there were no significant differences in any family at Romney at harvest. The combined analysis indicated that the wild-type and mutant plants were similar in biomass at Week 3 but the wild-type plants had a higher biomass than the C493Y mutants at Week 6 and at harvest.

Table 3.11 Biomass dry weights for the F_2 families measured at three sampling times at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni (Romney). The least squares means of the wild-type (WT) and acyanogenic mutant (C493Y) F_2 plants in each family were compared at each location and sampling time as well as averaged over locations and families. Differences (DIF) are considered significant if the P-value was less than 0.05. Significant (S) differences are highlighted in red and non-significant (NS) are not highlighted.

Biomass (grams)	Week 3			Week 6			HARVEST		
	WT	C493Y	DIF	WT	C493Y	DIF	WT	C493Y	DIF
ACRE									
2447 x 915B	2.2	2.3	NS	61.0	40.6	S	259.9	195.5	S
2447 x Tx623	1.9	1.6	NS	43.1	38.2	NS	139.9	134.1	NS
Tx2737 x 2447-1	2.7	2.3	NS	64.0	54.6	NS	133.8	123.9	NS
Tx2737 x 2447-2	2.3	1.9	NS	57.9	49.5	NS	134.2	107.6	S
ROMNEY									
2447 x 915B	1.9	1.8	NS	36.7	30.8	NS	218.6	218.3	NS
2447 x Tx623	2.9	2.4	NS	41.5	45.5	NS	117.6	103.6	NS
Tx2737 x 2447-1	2.0	1.7	NS	38.1	32.8	NS	104.3	115.7	NS
Tx2737 x 2447-2	1.8	2.0	NS	36.9	29.2	S	114.5	102.7	NS
AVERAGE	2.2	2.0	NS	47.4	40.5	S	152.5	136.8	S

Differences in grain yield between the wild-type and mutant plants are reported in (Table 3.12). Grain yield was significantly different between the wild-type and C493Y mutants in each family at both locations, with the exception of 2447 x 915B at Romney. Results from the combined analysis were similar with wild-type plants producing significantly higher yields than the C493Y mutants. The C493Y mutants produced about one-third less grain than the wild-type plants.

Table 3.12 Grain yield for the F_2 families measured at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni (Romney). The least squares means of the wild-type (WT) and acyanogenic mutant (C493Y) F_2 plants in each family

were compared at each location as well as averaged over locations and families. Differences (DIF) are considered significant if the P-value was less than 0.05. Significant (S) differences are highlighted in red and non-significant (NS) are not highlighted.

Grain Yield (gm)	Harvest		
	WT	C493Y	DIF
ACRE			
2447 x 915B	85.7	48.8	S
2447 x Tx623	75.3	47.1	S
Tx2737 x 2447-1	82.7	59.5	S
Tx2737 x 2447-2	73.0	47.0	S
ROMNEY			
2447 x 915B	74.7	75.8	NS
2447 x Tx623	81.1	45.0	S
Tx2737 x 2447-1	83.8	54.6	S
Tx2737 x 2447-2	74.8	58.0	S
AVERAGE	78.4	52.5	S

Differences in harvest index are shown in Table 3.13. Both 2447 x 915B and Tx2737 x 2447-2 did not show a significant difference in harvest index between the wild-type and C493Y mutants at either location. Tx2737 x 2447-1 exhibited a significant difference in harvest index between the groups at Romney but not at ACRE. 2447 x Tx623 showed a significant difference in harvest index between the wild-type and acyanogenic siblings at both locations. The combined analysis showed a similar result with the wild-type having a significantly greater harvest index than the C493Y mutants over locations.

Table 3.13 Harvest index for the F_2 families measured at Purdue University Agronomy Center for Research and Education (ACRE) and Ag Alumni (Romney). The least squares means of the wild-type (WT) and acyanogenic mutant (C493Y) F_2 plants in each family

were compared at each location as well as averaged over locations and families. Differences (DIF) are considered significant if the P-value was less than 0.05. Significant (S) differences are highlighted in red and non-significant (NS) are not highlighted.

Harvest Index	HARVEST		
	WT	C493Y	Sig.
ACRE			
2447 x 915B	0.25	0.22	NS
2447 x Tx623	0.35	0.25	S
Tx2737 x 2447-1	0.38	0.32	NS
Tx2737 x 2447-2	0.36	0.30	NS
ROMNEY			
2447 x 915B	0.26	0.30	NS
2447 x Tx623	0.41	0.29	S
Tx2737 x 2447-1	0.44	0.36	S
Tx2737 x 2447-2	0.40	0.36	NS
AVERAGE	0.35	0.30	S

3.3.3 Stay-Green under Drought Conditions

3.3.3.1 Dhurrin Concentration

BTx623 (wild-type), EMS 932 (*dhr2-1*), an F_3 selection from EMS 5085 x Tx623 (*Cyp79A1-2*), and an acyanogenic check (maize) were grown on sand benches and leaf samples were taken from seedlings for quantification of dhurrin content in these lines. The results are presented below in Table 3.14. EMS 932 had the highest dhurrin concentration followed by BTx623 with EMS 5085 x Tx623 having the lowest dhurrin concentration similar to the maize check. Because maize does not produce dhurrin, we can assume that the values provided for maize are a base level of HPLC retention time and the acyanogenic EMS5085 x Tx623 F_3 population does not produce dhurrin either.

Table 3.14 Mean dhurrin concentration ($\mu g/cm^2$) values for lines BTx623 (wild-type), EMS 932 (*dhr2-1*), an F_3 selection from EMS 5085 x Tx623 (*Cyp79A1-2*), and an acyanogenic check (maize). The statistic groupings are based on a Waller-Duncan *k*-ratio *t*-test with a minimum significant value of 3.75.

Statistic Grouping	Pedigree	Dhurrin ($\mu g/cm^2$)	Sample Size
A	EMS 932	16.4	3
B	BTx623	9.5	3
C	Check (Maize)	3.4	3
C	EMS 5085 x Tx623	1.8	3

3.3.3.2   Characterization of Stay-Green

Chlorophyll content index means over time are shown in Figure 3.2. Sampling was initiated three weeks after planting. The CCI means of the three lines were not significantly different from each other from Weeks 3 to 6 before the drought treatment was imposed. Beginning at Weeks 7 and 8, BTx623 exhibited a significantly higher CCI than either EMS 5085 F_3 or EMS 932. At Week 9, EMS 5085 F_3 and BTx623 had a significantly higher CCI than EMS 932. Drought treatment was applied after Week 9. At Week 10, both the well-watered and drought-stressed treatments for EMS 5085 F_3 and BTx623 were statistically similar and higher than the drought and irrigated treatments of EMS 932. At Week 11, the well-watered treatments for EMS 5085 F_3 and BTx623 were statistically similar; however, the drought-treatment for BTx623 exhibited a significantly higher CCI value than either the drought-stressed EMS 932 or EMS 5085 F_3. At Week 12, all lines had begun to senesce and there were no significant differences between any pedigree and watering treatment combination.
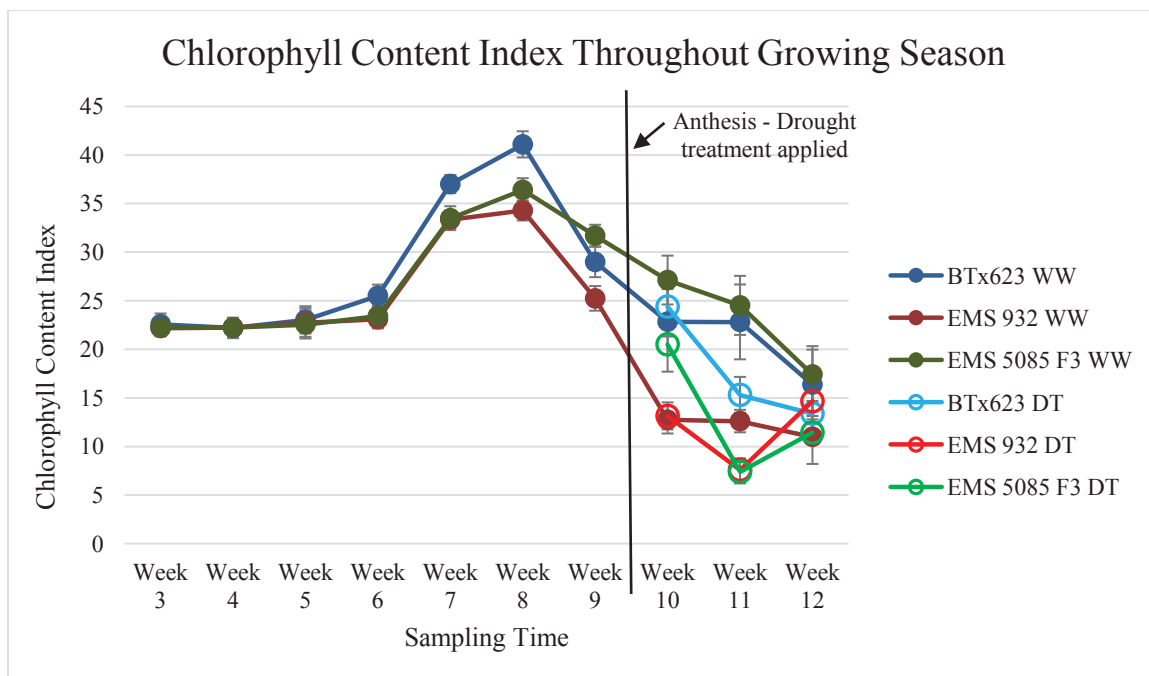
Figure 3.2 Chlorophyll content index (CCI) of BTx623, EMS 932, and EMS 5085 F3 expressed over time by pedigree and watering regimen, either well-watered (WW) or drought-stressed (DT). Sampling initiated three weeks after planting. Drought stress was applied when 90% of plants had reached anthesis. Standard error bars are provided for each pedigree and watering regimen.

Volumetric water content (VWC) of the plant was measured to verify that the soil conditions of the two water treatments were significantly different from each other. The results are presented below in Table 3.15. At all sampling times, the VWC for the well-watered treatment was significantly higher than the drought-stress treatment, indicating that the drought-stressed plants were experiencing different soil moisture conditions than the well-watered treatment.

Table 3.15 Volumetric water content (VWC) means in percent (%) for the well-watered (WW) and drought (DT) treatments. The treatments were compared by a least significant

difference value (LSD) calculated by the ANOVA procedure in SAS and whether the differences were significant (S) or not significant (NS).

VWC Means	WW	DT	LSD	Sig.
Week 10	30.3	17.1	2.42	S
Week 11	21.9	11.6	2.02	S
Week 12	43.5	23.7	1.99	S

Relative water content of the plants was measured and calculated by the formula described in Section 3.2.5. However, due to an error with the scale, the results of the RWC measurements were sometimes negative percentages, or percentages above 100%, neither of which is accurate for this type of measurement. These inconclusive results were not included.

3.4 Discussion

The goal of this research was to determine if lack of dhurrin production had any effects on growth and development of sorghum. Blomstedt et al. (2012) developed an EMS mutant sorghum population and identified a line that lacked dhurrin production due to a P414L mutation in CYP79A1. They noted that this acyanogenic mutant exhibited slightly slower growth in the seedling stage but did not determine in any detail the effects of the mutation on growth and development characteristics. In order to identify if any delays in growth and development existed in acyanogenic plants, we collected several phenotypic measurements throughout the growing season in F_2 families segregating for wild-type and the dhurrin-free C493Y mutation.

Very few differences in chlorophyll content were observed when comparing wild-type and dhurrin-free plants throughout the growing season. These results suggest that the mutant plants had sufficient N content for normal growth. Several studies have suggested that dhurrin may be an important nitrogen storage pool (McBee and Miller, 1980; Busk and Møller, 2002; Jenrich et al., 2007). If dhurrin played a central role in N-metabolism of sorghum, one might expect to observe N-deficiency in mutant plants but we did not observe these symptoms in the dhurrin-free mutant. However, these plants were not under drought-stressed conditions, when differences in stay-green are more evident. The plants also received adequate fertility conditions. Borrell and Hammer (2000) demonstrate the positive relationship between stay-green and leaf N concentration. A future experiment with these families under optimum and low N fertility conditions could provide more insight into dhurrin's relationship with nitrogen metabolism.

Significant differences in plant height and biomass were observed when comparing mutant and wild-type F_2 plants at ACRE and once at Romney. These differences were more pronounced at maturity and seemed to associate with reduced grain yield in the mutant plants. These results are different from those reported by Blomstedt et al. (2013) who observed slower seedling growth of their CYP79A1 mutant line compared to wild-type plants.

Grain yield for all families demonstrated significant difference between the wild-type and acyanogenic siblings at both locations with the exception of 2447 x 915B at Romney. This lag in yield in the dhurrin-free siblings could be due to the genetic load caused by the other EMS mutations in the background. Perhaps a closely linked mutation is affecting seed number or seed size. Dhurrin is also believed to be a defense compound

against herbivory (Morant et al., 2003; Kristensen et al., 2005; Krothapalli et al., 2013). Perhaps the loss of dhurrin in this capacity for defense could be a contributing factor in the differences observed in biomass or grain yield.

Chlorophyll content is related to nitrogen status of the plant. Burke et al. (2013) suggested that dhurrin content in the seedling is a predictor of stay-green during anthesis. It would be assumed that wild-type plants containing dhurrin would have more chlorophyll or stay-green capacity than their acyanogenic, mutant type counterparts. In this study, there were few statistically significant differences in chlorophyll content in the F_2 families throughout the field experiment.

In the drought experiment, chlorophyll content of the wild-type plants was higher than the mutant-types before and after the drought stress treatment was applied. In this instance, it would appear that Burke et al. (2013) was correct in the positive relationship between dhurrin concentration and stay-green. O'Donnell et al. (2013) used chemically induced osmotic stress to simulate drought and verified that dhurrin concentration increased after drought stress, partly due to stunted plant growth. It can be argued that the wild-type line used in this experiment is not considered a stay-green genotype. For our interests in identifying the underlying genetic cause of the mutations, it was important to use the reference genome. Future studies could incorporate the C493Y mutation into a stay-green background and compare F_2 populations from that cross. Since there are many other EMS mutations in the background, the genetic load could also have an effect on stay-green. If the background mutations could be cleaned up, future studies could focus directly on the effects of the C493Y mutation by eliminating any interference created by the genetic load. Another concern that was not addressed in this particular experiment

was the performance of the acyanogenic mutant under nitrogen deficient conditions, since all plants received ideal fertility treatments in both experiments. This could be another area for future research to better understand the effects of lack of dhurrin of sorghum growth and development as well as stay-green.

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APPENDICES

Appendix A   SAS Code

1. <u>Sample SAS Code for Parent Line Growth and Development Characteristics</u>

This is the SAS code used to analyze the parents' growth and development phenotype

data at the harvest sampling time.

```
**Entering the data file into SAS**;

data HARVESTPARENTS;

infile 'Dhurrin Experiment HARVEST PARENTS.csv' dsd firstobs=2 missover;

length Pedigree $19 ;

input Plot $ Row $ Range $ Location $ Pedigree $ HCNTest $ Chlorophyll Inches Height

Grain Head Stalk TotalBiomass HarvestIndex; run;

proc print data=HARVESTPARENTS; run;

*****CHLOROPHYLL*****;

proc anova data=HARVESTPARENTS;

class Pedigree;

by Location;

model Chlorophyll=Pedigree;

means Pedigree/ lsd; run;

*****HEIGHT*****;

proc anova data=HARVESTPARENTS;

class Pedigree;

by Location;
```

```
model Height=Pedigree;

means Pedigree/ lsd; run;

*****GRAIN YIELD*****;

proc anova data=HARVESTPARENTS;

class Pedigree;

by Location;

model Grain=Pedigree;

means Pedigree/ lsd; run;

*****TOTAL BIOMASS*****;

proc anova data=HARVESTPARENTS;

class Pedigree;

by Location;

model TotalBiomass=Pedigree;

means Pedigree/ lsd; run;

*****Harvest Index*****;

proc anova data=HARVESTPARENTS;

class Pedigree;

by Location;

model HarvestIndex=Pedigree;

means Pedigree/ lsd; run;
```

2. <u>Sample SAS Code for F_2 Families Growth and Development Characteristics</u>

This is the SAS code for the 2447 x 915B F_2 family for all of the phenotypes measured at the harvest sampling time. The same code was used for the other F_2 families, changing the data name and infile according to the name of the file for each family.

```
**Entering the data file into SAS**;
data F2915B ;
infile 'Dhurrin Experiment Harvest 915B with location data.csv' dsd firstobs=2 missover;
length Pedigree $19;
input Plot $ Row $ Range $ Location $ Pedigree $ HCNTest $ Chlorophyll INCHES
Height GrainYield Headweight Biomass TotalBio HarvestIndex;
 if N(Chlorophyll, Height, GrainYield, TotalBio, HarvestIndex) ne 5 then PUT 'Missing
data found ' Plot Location HCNTest Chlorophyll INCHES Height GrainYield TotalBio
HarvestIndex; run;
proc sort data=F2915B;
by Location;
proc print data=F2915B; run;
proc glm data=F2915B ;
by Location;
class Plot HCNTest;
model Chlorophyll Height GrainYield TotalBio HarvestIndex= Plot HCNTest/ ss3;
lsmeans HCNTest / pdiff ; run;
```

3. Sample SAS Code for Stay-Green and Drought Experiment

```
**Entering the data file into SAS**;

data Sample9;

infile 'Three Line GH Exp Sample 9.csv' dsd firstobs=2 missover;

length Line $23;

input Rep Line $ WaterTreatment $ Chlorophyll VWC PER Code $; run;

proc print data=Sample9; run;

proc sort data=Sample9;

by Code; run;

**Calculate the means and standard error**;

proc means data=Sample9;

by Code;

var Chlorophyll;

title 'Chlorophyll Means Sample 9';

output out=meansout mean=mean stderr=stderr; run;

**Print out the values for the standard deviation**;

data reshape (drop=stderr);

set=meansout;

lower= - stderr ;

upper= + stderr; run;

proc print data=reshape; run;

**Compares all means to each other**;

proc anova data=Sample9;
```

```
class Code;

model Chlorophyll=Code;

means Code/ tukey;

title 'Chlorophyll ANOVA and LSD Sample 9'; run;

**Compares the means to a least significant difference**;

proc anova data=Sample9;

class Code;

model Chlorophyll=Code;

means Code/ duncan waller;

title 'Chlorophyll ANOVA and LSD Sample 9'; run;

proc anova data=Sample9;

class WaterTreatment;

model VWC=WaterTreatment;

means WaterTreatment/ lsd;

title 'VWC ANOVA and LSD Sample 9'; run;

proc anova data=Sample9;

class WaterTreatment;

model PER=WaterTreatment;

means WaterTreatment/ lsd;

title 'PER ANOVA and LSD Sample 9'; run;
```

4. Sample SAS Code for Dhurrin Concentration Analysis

```
data DhurrinData;

keep Sample Plot Pedigree HCNTest Code Dhurrin;

infile 'Dhurrin Content for Field Plots.csv' dsd firstobs=2 missover;

length Pedigree $17 ;

input Sample Plot Pedigree $ HCNTest $ Code $ LineID $ Dhurrin Fructose Glucose

Sucrose Area Extract Leaf Dried Equivalent Resuspension HPLC Injection Analyzed D F

G S; run;

proc print data=DhurrinData; run;

proc means data=DhurrinData nway noprint;

class Plot Code;

var Dhurrin;

output out=DhurrinPlots N=N Mean= ; run;

*****Compares all means to each other****;

proc glm data=DhurrinPlots;

class Code;

model Dhurrin=Code;

means Code;

means Code/ tukey;

lsmeans Code / pdiff; quit;
```

Appendix B   Data Files

1.   <u>Growth and Development Experiment Week 3 Sampling Data</u>

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9017	Tx2737 x EMS 2447-1	W	15.7	9	22.86	6	2.47
9017	Tx2737 x EMS 2447-1	B	17.7	10	25.4	5	1.38
9018	Tx2737 x EMS 2447-1	W	32.9	11	27.94	6	3.10
9018	Tx2737 x EMS 2447-1	B	29.7	12	30.48	6	2.94
9019	Tx2737 x EMS 2447-1	W	20.4	11	27.94	5	1.94
9019	Tx2737 x EMS 2447-1	B	22.7	10	25.4	6	2.36
9020	Tx2737 x EMS 2447-1	W	25.1	11	27.94	5	2.24
9020	Tx2737 x EMS 2447-1	B	21.9	12	30.48	6	2.96
9021	Tx2737 x EMS 2447-1	W	.	.	.	.	.
9021	Tx2737 x EMS 2447-1	B	35.5	14	35.56	7	4.56
9022	Tx2737 x EMS 2447-1	W	29.2	12	30.48	5	2.27
9022	Tx2737 x EMS 2447-1	B	31.2	12	30.48	5	2.21
9023	Tx2737 x EMS 2447-1	W	31.8	12	30.48	5	3.21
9023	Tx2737 x EMS 2447-1	B	28.1	12	30.48	5	2.59
9024	Tx2737 x EMS 2447-1	W	32.6	11	27.94	5	2.62
9024	Tx2737 x EMS 2447-1	B	20.9	10	25.4	5	1.22
9025	Tx2737 x EMS 2447-1	W	25.3	13	33.02	6	3.01
9025	Tx2737 x EMS 2447-1	B	35.2	12	30.48	6	3.91
9026	Tx2737 x EMS 2447-1	W	17.8	12	30.48	6	2.39
9026	Tx2737 x EMS 2447-1	B	29.9	15	38.1	5	3.75
9027	Tx2737 x EMS 2447-1	W	23.3	10	25.4	5	1.38
9027	Tx2737 x EMS 2447-1	B	18.5	9	22.86	5	0.94
9028	Tx2737 x EMS 2447-1	W	21.5	12	30.48	6	1.62
9028	Tx2737 x EMS 2447-1	B	26.2	15	38.1	6	3.20
9029	Tx2737 x EMS 2447-1	W	29.8	12	30.48	6	2.58
9029	Tx2737 x EMS 2447-1	B	27.2	12	30.48	6	3.22
9030	Tx2737 x EMS 2447-1	W	28.3	11	27.94	5	1.42
9030	Tx2737 x EMS 2447-1	B	22.6	15	38.1	7	4.64
9031	Tx2737 x EMS 2447-1	W	25.2	9	22.86	5	1.03
9031	Tx2737 x EMS 2447-1	B	28.0	8	20.32	6	1.34
9032	Tx2737 x EMS 2447-1	W	25.1	11	27.94	5	1.96
9032	Tx2737 x EMS 2447-1	B	19.9	11	27.94	7	1.97
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9117	Tx2737 x EMS 2447-1	B	33.9	14	35.56	6	4.19

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9117	Tx2737 x EMS 2447-1	W	43.0	12	30.48	5	3.55
9118	Tx2737 x EMS 2447-1	B	35.7	14	35.56	5	3.94
9118	Tx2737 x EMS 2447-1	W	34.9	12	30.48	5	2.42
9119	Tx2737 x EMS 2447-1	B	16.1	9	22.86	5	0.94
9119	Tx2737 x EMS 2447-1	W	34.6	12	30.48	5	2.22
9120	Tx2737 x EMS 2447-1	B	28.2	10	25.4	5	1.58
9120	Tx2737 x EMS 2447-1	W	33.8	10	25.4	4	1.19
9121	Tx2737 x EMS 2447-1	B	36.8	14	35.56	7	4.06
9121	Tx2737 x EMS 2447-1	W	45.2	13	33.02	6	3.67
9122	Tx2737 x EMS 2447-1	B	22.8	10	25.4	6	1.36
9122	Tx2737 x EMS 2447-1	W	34.9	12	30.48	6	3.10
9123	Tx2737 x EMS 2447-1	B	24.5	11	27.94	6	2.12
9123	Tx2737 x EMS 2447-1	W	28.2	10	25.4	5	0.65
9124	Tx2737 x EMS 2447-1	B	27.9	9	22.86	5	0.83
9124	Tx2737 x EMS 2447-1	W	30.7	8	20.32	5	1.50
9125	Tx2737 x EMS 2447-1	B	38.9	10	25.4	6	2.30
9125	Tx2737 x EMS 2447-1	W	.	.	.	.	.
9126	Tx2737 x EMS 2447-1	B	24.6	9	22.86	5	1.94
9126	Tx2737 x EMS 2447-1	W	22.2	7	17.78	4	0.49
9127	Tx2737 x EMS 2447-1	B	22.8	7	17.78	5	0.78
9127	Tx2737 x EMS 2447-1	W	39.4	8	20.32	5	1.07
9128	Tx2737 x EMS 2447-1	B	26.7	9	22.86	5	1.15
9128	Tx2737 x EMS 2447-1	W	22.6	7	17.78	5	0.73
9129	Tx2737 x EMS 2447-1	B	20.3	9	22.86	6	1.09
9129	Tx2737 x EMS 2447-1	W	23.4	6	15.24	5	0.36
9130	Tx2737 x EMS 2447-1	B	43.2	12	30.48	6	2.10
9130	Tx2737 x EMS 2447-1	W	31.9	8	20.32	6	0.96
9131	Tx2737 x EMS 2447-1	B	28.6	9	22.86	6	1.29
9131	Tx2737 x EMS 2447-1	W	24.8	10	25.4	6	1.35
9132	Tx2737 x EMS 2447-1	B	39.7	12	30.48	6	2.70
9132	Tx2737 x EMS 2447-1	W	26.6	10	25.4	6	1.49
9033	Tx2737 x EMS 2447-2	W	26.4	12	30.48	5	2.57
9033	Tx2737 x EMS 2447-2	B	19.5	11	27.94	5	1.89
9034	Tx2737 x EMS 2447-2	W	23.8	10	25.4	6	2.27
9034	Tx2737 x EMS 2447-2	B	22.4	12	30.48	6	2.52
9035	Tx2737 x EMS 2447-2	W	19.2	9	22.86	5	1.00
9035	Tx2737 x EMS 2447-2	B	21.8	10	25.4	5	1.25
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9036	Tx2737 x EMS 2447-2	W	32.0	11	27.94	5	1.89

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9036	Tx2737 x EMS 2447-2	B	24.3	13	33.02	6	2.89
9037	Tx2737 x EMS 2447-2	W	33.6	14	35.56	6	4.22
9037	Tx2737 x EMS 2447-2	B	30.9	13	33.02	6	3.77
9038	Tx2737 x EMS 2447-2	W	23.3	13	33.02	6	3.07
9038	Tx2737 x EMS 2447-2	B	23.2	13	33.02	6	3.19
9039	Tx2737 x EMS 2447-2	W	34.5	12	30.48	6	2.73
9039	Tx2737 x EMS 2447-2	B	37.3	12	30.48	6	2.98
9040	Tx2737 x EMS 2447-2	W	19.5	8	20.32	5	0.62
9040	Tx2737 x EMS 2447-2	B	20.3	11	27.94	5	1.39
9041	Tx2737 x EMS 2447-2	W	23.7	8	20.32	4	0.83
9041	Tx2737 x EMS 2447-2	B	26.0	16.	40.64	7	3.28
9042	Tx2737 x EMS 2447-2	W	21.9	9	22.86	4	0.79
9042	Tx2737 x EMS 2447-2	B	20.6	10	25.4	5	1.13
9043	Tx2737 x EMS 2447-2	W	20.2	8	20.32	5	1.33
9043	Tx2737 x EMS 2447-2	B	24.5	11	27.94	5	2.28
9044	Tx2737 x EMS 2447-2	W	24.9	9	22.86	5	1.64
9044	Tx2737 x EMS 2447-2	B	22.8	10	25.4	6	1.26
9045	Tx2737 x EMS 2447-2	W	25.9	11	27.94	6	2.38
9045	Tx2737 x EMS 2447-2	B	26.4	12	30.48	6	2.04
9046	Tx2737 x EMS 2447-2	W	20.0	13	33.02	6	2.85
9046	Tx2737 x EMS 2447-2	B	27.0	12	30.48	7	3.33
9047	Tx2737 x EMS 2447-2	W	26.0	10	25.4	6	1.59
9047	Tx2737 x EMS 2447-2	B	23.0	9	22.86	6	1.33
9048	Tx2737 x EMS 2447-2	W	16.2	7	17.78	5	0.37
9048	Tx2737 x EMS 2447-2	B	22.5	11	27.94	6	1.55
9133	Tx2737 x EMS 2447-2	B	30.8	12	30.48	5	2.29
9133	Tx2737 x EMS 2447-2	W	42.2	12	30.48	5	2.26
9134	Tx2737 x EMS 2447-2	B	27.9	14	35.56	6	1.81
9134	Tx2737 x EMS 2447-2	W	33.8	14	35.56	6	4.26
9135	Tx2737 x EMS 2447-2	B	42.0	10	25.4	5	1.57
9135	Tx2737 x EMS 2447-2	W	17.4	10	25.4	5	1.48
9136	Tx2737 x EMS 2447-2	B	38.6	12	30.48	6	2.80
9136	Tx2737 x EMS 2447-2	W	31.3	11	27.94	6	1.51
9137	Tx2737 x EMS 2447-2	B	29.4	17	43.18	7	4.05
9137	Tx2737 x EMS 2447-2	W	42.9	17	43.18	7	4.10
9138	Tx2737 x EMS 2447-2	B	28.4	10	25.4	6	1.60
9138	Tx2737 x EMS 2447-2	W	31.9	11	27.94	6	1.91
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9139	Tx2737 x EMS 2447-2	B	29.1	10	25.4	5	1.18

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9139	Tx2737 x EMS 2447-2	W	30.6	13	33.02	7	3.99
9140	Tx2737 x EMS 2447-2	B	34.5	11	27.94	5	1.70
9140	Tx2737 x EMS 2447-2	W	28.0	8	20.32	5	0.82
9141	Tx2737 x EMS 2447-2	B	28.5	15	38.1	6	2.42
9141	Tx2737 x EMS 2447-2	W	32.1	12	30.48	6	2.39
9142	Tx2737 x EMS 2447-2	B	24.9	10	25.4	5	1.07
9142	Tx2737 x EMS 2447-2	W	25.3	11	27.94	6	1.20
9143	Tx2737 x EMS 2447-2	B	31.3	11	27.94	6	2.45
9143	Tx2737 x EMS 2447-2	W	31.5	9	22.86	6	1.23
9144	Tx2737 x EMS 2447-2	B	26.1	11	27.94	5	1.49
9144	Tx2737 x EMS 2447-2	W	26.3	10	25.4	5	1.68
9145	Tx2737 x EMS 2447-2	B	31.3	7	17.78	5	0.67
9145	Tx2737 x EMS 2447-2	W	34.4	8	20.32	5	0.49
9146	Tx2737 x EMS 2447-2	B	46.3	10	25.4	6	1.97
9146	Tx2737 x EMS 2447-2	W	32.6	10	25.4	5	1.52
9147	Tx2737 x EMS 2447-2	B	21.1	9	22.86	5	0.93
9147	Tx2737 x EMS 2447-2	W	30.1	11	27.94	6	2.07
9148	Tx2737 x EMS 2447-2	B	29.2	10	25.4	6	1.36
9148	Tx2737 x EMS 2447-2	W	41.0	7	17.78	5	0.55
9149	EMS 2447 x 915B	B	31.9	11	28	5	1.60
9149	EMS 2447 x 915B	W	35.2	13	33	5	2.55
9150	EMS 2447 x 915B	B	34.0	11	28	5	1.14
9150	EMS 2447 x 915B	W	30.7	11	28	5	1.12
9151	EMS 2447 x 915B	B	32.5	15	38	6	2.88
9151	EMS 2447 x 915B	W	31.0	16	41	6	3.56
9152	EMS 2447 x 915B	B	36.5	13	33	6	2.22
9152	EMS 2447 x 915B	W	40.5	8	20	5	0.97
9153	EMS 2447 x 915B	B	34.3	10	25	6	1.44
9153	EMS 2447 x 915B	W	29.6	10	25	5	0.98
9154	EMS 2447 x 915B	B	30.5	13	33	5	1.68
9154	EMS 2447 x 915B	W	28.5	14	36	6	3.26
9155	EMS 2447 x 915B	B	38.4	10	25	5	2.14
9155	EMS 2447 x 915B	W	34.0	10	25	5	1.51
9156	EMS 2447 x 915B	B	32.5	10	25	5	1.05
9156	EMS 2447 x 915B	W	34.9	15	38	6	3.12
9157	EMS 2447 x 915B	B	37.0	13	33	5	2.71
9157	EMS 2447 x 915B	W	54.4	11	28	6	1.77
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9158	EMS 2447 x 915B	B	27.1	10	25	5	1.61

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9158	EMS 2447 x 915B	W	37.0	10	25	5	1.25
9159	EMS 2447 x 915B	B	24.4	11	28	5	1.40
9159	EMS 2447 x 915B	W	22.7	7	18	5	0.47
9160	EMS 2447 x 915B	B	31.3	12	30	6	2.02
9160	EMS 2447 x 915B	W	23.9	9	23	5	1.04
9161	EMS 2447 x 915B	B	44.6	10	25	6	1.45
9161	EMS 2447 x 915B	W	27.9	10	25	6	1.61
9162	EMS 2447 x 915B	B	40.6	10	25	6	2.37
9162	EMS 2447 x 915B	W	28.8	12	30	6	2.95
9163	EMS 2447 x 915B	B	28.1	10	25	5	1.27
9163	EMS 2447 x 915B	W	24.3	10	25	5	1.72
9164	EMS 2447 x 915B	B	45.1	13	33	5	2.69
9164	EMS 2447 x 915B	W	27.7	10	25	5	1.71
9049	EMS 2447 x 915B	W	18.1	15	38	6	3.52
9049	EMS 2447 x 915B	B	19.3	11	28	5	3.22
9050	EMS 2447 x 915B	W	27.2	12	30	6	2.86
9050	EMS 2447 x 915B	B	33.8	11	28	5	1.87
9051	EMS 2447 x 915B	W	32.1	12	30	5	2.92
9051	EMS 2447 x 915B	B	23.9	12	30	5	2.20
9052	EMS 2447 x 915B	W	19.8	10	25	5	1.31
9052	EMS 2447 x 915B	B	26.0	12	30	5	2.64
9053	EMS 2447 x 915B	W	27.6	10	25	5	1.27
9053	EMS 2447 x 915B	B	19.0	13	33	5	2.29
9054	EMS 2447 x 915B	W	21.8	13	33	5	2.41
9054	EMS 2447 x 915B	B	22.9	12	30	6	2.29
9055	EMS 2447 x 915B	W	27.7	12	30	5	1.94
9055	EMS 2447 x 915B	B	24.7	12	30	5	1.81
9056	EMS 2447 x 915B	W	25.0	11	28	5	2.06
9056	EMS 2447 x 915B	B	26.8	12	30	5	1.76
9057	EMS 2447 x 915B	W	28.6	13	33	6	2.19
9057	EMS 2447 x 915B	B	23.9	12	30	5	1.62
9058	EMS 2447 x 915B	W	26.4	14	36	5	2.64
9058	EMS 2447 x 915B	B	23.6	11	28	5	1.70
9059	EMS 2447 x 915B	W	24.6	11	28	5	1.37
9059	EMS 2447 x 915B	B	17.0	11	28	6	1.77
9060	EMS 2447 x 915B	W	31.9	13	33	6	2.71
9060	EMS 2447 x 915B	B	18.0	15	38	6	3.02
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9061	EMS 2447 x 915B	W	30.9	12	30	5	1.29

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9061	EMS 2447 x 915B	B	28.6	11	28	5	1.80
9062	EMS 2447 x 915B	W	32.8	11	28	5	2.75
9062	EMS 2447 x 915B	B	20.8	14	36	6	2.69
9063	EMS 2447 x 915B	W	39.3	10	25	6	1.25
9063	EMS 2447 x 915B	B	23.6	10	25	6	1.68
9064	EMS 2447 x 915B	W	33.1	13	33	7	3.95
9064	EMS 2447 x 915B	B	18.9	12	30	6	2.31
9065	EMS 2447 x Tx623	W	30.7	12	30.48	5	2.90
9065	EMS 2447 x Tx623	B	32.6	12	30.48	5	3.21
9066	EMS 2447 x Tx623	W	26.7	14	35.56	6	3.16
9066	EMS 2447 x Tx623	B	17.3	12	30.48	5	2.23
9067	EMS 2447 x Tx623	W	18.2	9	22.86	5	1.54
9067	EMS 2447 x Tx623	B	28.2	11	27.94	5	1.25
9068	EMS 2447 x Tx623	W	31.9	10	25.4	5	1.48
9068	EMS 2447 x Tx623	B	26.1	12	30.48	6	2.84
9069	EMS 2447 x Tx623	W	33.7	11	27.94	5	1.95
9069	EMS 2447 x Tx623	B	22.5	12	30.48	5	1.76
9070	EMS 2447 x Tx623	W	24.0	12	30.48	5	1.59
9070	EMS 2447 x Tx623	B	28.9	14	35.56	6	3.65
9071	EMS 2447 x Tx623	W	19.6	7	17.78	4	0.42
9071	EMS 2447 x Tx623	B	21.6	10	25.4	5	1.71
9072	EMS 2447 x Tx623	W	24.3	10	25.4	5	1.32
9072	EMS 2447 x Tx623	B	19.3	11	27.94	5	1.68
9073	EMS 2447 x Tx623	W	20.2	8	20.32	4	0.71
9073	EMS 2447 x Tx623	B	21.8	11	27.94	3	1.84
9074	EMS 2447 x Tx623	W	30.7	9	22.86	5	2.54
9074	EMS 2447 x Tx623	B	27.3	10	25.4	5	2.58
9075	EMS 2447 x Tx623	W	23.8	9	22.86	5	0.85
9075	EMS 2447 x Tx623	B	27.3	10	25.4	5	1.38
9076	EMS 2447 x Tx623	W	19.2	5	12.7	4	0.17
9076	EMS 2447 x Tx623	B	16.5	7	17.78	5	0.58
9077	EMS 2447 x Tx623	W	19.7	10	25.4	5	1.00
9077	EMS 2447 x Tx623	B	20.2	10	25.4	5	1.15
9078	EMS 2447 x Tx623	W	18.4	11	27.94	6	2.07
9078	EMS 2447 x Tx623	B	19.2	9	22.86	6	1.35
9079	EMS 2447 x Tx623	W	28.7	13	33.02	6	3.11
9079	EMS 2447 x Tx623	B	28.1	11	27.94	6	1.44
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9080	EMS 2447 x Tx623	W	16.0	6	15.24	5	0.45

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9080	EMS 2447 x Tx623	B	25.7	11	27.94	5	1.72
9165	EMS 2447 x Tx623	B	40.4	16	40.64	5	3.08
9165	EMS 2447 x Tx623	W	30.9	12	30.48	5	2.00
9166	EMS 2447 x Tx623	B	33.3	13	33.02	6	3.01
9166	EMS 2447 x Tx623	W	43.8	13	33.02	5	3.38
9167	EMS 2447 x Tx623	B	39.8	13	33.02	6	3.51
9167	EMS 2447 x Tx623	W	38.7	15	38.1	6	3.74
9168	EMS 2447 x Tx623	B	26.6	14	35.56	6	2.28
9168	EMS 2447 x Tx623	W	41.9	14	35.56	6	3.04
9169	EMS 2447 x Tx623	B	40.8	14	35.56	6	3.99
9169	EMS 2447 x Tx623	W	28.0	14	35.56	6	2.58
9170	EMS 2447 x Tx623	B	30.7	14	35.56	6	2.38
9170	EMS 2447 x Tx623	W	30.1	9	22.86	5	1.23
9171	EMS 2447 x Tx623	B	33.2	12	30.48	5	1.52
9171	EMS 2447 x Tx623	W	43.2	12	30.48	6	2.09
9172	EMS 2447 x Tx623	B	25.8	10	25.4	6	1.41
9172	EMS 2447 x Tx623	W	27.9	10	25.4	5	1.60
9173	EMS 2447 x Tx623	B	32.4	11	27.94	6	1.82
9173	EMS 2447 x Tx623	W	34.7	11	27.94	6	2.38
9174	EMS 2447 x Tx623	B	36.7	13	33.02	6	3.12
9174	EMS 2447 x Tx623	W	35.7	12	30.48	6	2.87
9175	EMS 2447 x Tx623	B	34.7	10	25.4	6	2.49
9175	EMS 2447 x Tx623	W	34.8	10	25.4	6	2.34
9176	EMS 2447 x Tx623	B	29.3	12	30.48	6	2.38
9176	EMS 2447 x Tx623	W	38.5	11	27.94	6	2.14
9177	EMS 2447 x Tx623	B	38.3	13	33.02	7	3.96
9177	EMS 2447 x Tx623	W	34.6	13	33.02	6	2.80
9178	EMS 2447 x Tx623	B	34.3	12	30.48	6	3.78
9178	EMS 2447 x Tx623	W	33.9	13	33.02	6	3.04
9179	EMS 2447 x Tx623	B	37.9	12	30.48	6	3.42
9179	EMS 2447 x Tx623	W	34.1	9	22.86	5	1.31
9180	EMS 2447 x Tx623	B	32.8	12	30.48	6	3.94
9180	EMS 2447 x Tx623	W	29.3	8	20.32	5	1.15
9001	EMS 2447 mut	W	20.5	10	25.4	5	1.21
9002	Tx2737	B	25.5	11	27.94	5	1.76
9003	B Tx623	B	24.6	12	30.48	5	1.81
9004	915 B	B	18.9	11	27.94	5	1.23
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9005	915 B	B	16.6	12	30.48	5	1.76

9006	EMS 2447 mut	W	28.7	11	27.94	5	1.84
9007	B Tx623	B	28.6	12	30.48	6	2.65
9008	Tx2737	B	16.1	11	27.94	5	1.55
9009	B Tx623	B	26.9	12	30.48	6	2.29
9010	915 B	B	19.5	13	33.02	4	2.66
9011	EMS 2447 mut	W	25.4	11	27.94	5	1.91
9012	Tx2737	B	17.4	12	30.48	6	2.44
9013	EMS 2447 mut	W	20.8	11	27.94	4	2.22
9014	B Tx623	B	31.1	13	33.02	6	2.93
9015	Tx2737	B	24.0	13	33.02	6	3.65
9016	915 B	B	20.4	14	35.56	5	2.72
9101	B Tx623	B	30.0	9	22.86	5	0.97
9102	915 B	B	32.9	12	30.48	5	1.72
9103	Tx2737	B	20.3	10	25.4	5	1.03
9104	EMS 2447 mut	W	38.6	10	25.4	4	0.99
9105	915 B	B	25.3	9	22.86	4	0.62
9106	Tx2737	B	35.1	8	20.32	4	0.67
9107	EMS 2447 mut	W	22.8	8	20.32	4	0.54
9108	B Tx623	B	31.9	11	27.94	4	1.08
9109	915 B	B	30.5	12	30.48	5	2.24
9110	EMS 2447 mut	W	21.2	9	22.86	5	0.91
9111	Tx2737	B	20.5	7	17.78	4	0.46
9112	B Tx623	B	35.0	9	22.86	4	0.75
9113	Tx2737	B	38.1	12	30.48	5	1.93
9114	915 B	B	36.2	12	30.48	5	1.60
9115	EMS 2447 mut	W	28.6	12	30.48	5	2.55
9116	B Tx623	B	42.2	14	35.56	5	3.12

2. Growth and Development Experiment Week 6 Sampling Data

Plot	Pedigree	FA	Chlorophyll	Ht.	Height	Leaf	Biomass

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9001	EMS 2447 mut	W	38.4	20	50.80	7	14.71
9002	Tx2737	B	50.4	26	66.04	8	48.26
9003	B Tx623	B	40.9	34	86.36	8	49.73
9004	915 B	B	38.5	27	68.58	7	33.63
9005	915 B	B	37.3	28	71.12	7	36.35
9006	EMS 2447 mut	W	39.4	29	73.66	8	21.17
9007	B Tx623	B	37.7	34	86.36	8	32.39
9008	Tx2737	B	42.4	28	71.12	8	21.28
9009	B Tx623	B	42.1	28	71.12	7	25.09
9010	915 B	B	43.2	31	78.74	7	58.19
9011	EMS 2447 mut	W	40.0	22	55.88	8	12.09
9012	Tx2737	B	52.1	32	81.28	9	33.07
9013	EMS 2447 mut	W	51.5	29	73.66	8	41.39
9014	B Tx623	B	28.9	33	83.82	9	45.55
9015	Tx2737	B	56.5	28	71.12	9	50.48
9016	915 B	B	38.6	34	86.36	8	60.00
9101	B Tx623	B	31.8	31	78.74	8	23.81
9102	915 B	B	44.9	32	81.28	7	22.17
9103	Tx2737	B	47.1	27	68.58	8	16.77
9104	EMS 2447 mut	W	71.5	25	63.50	9	23.06
9105	915 B	B	43.4	32	81.28	8	20.20
9106	Tx2737	B	52.9	29	73.66	10	32.80
9107	EMS 2447 mut	W	48.8	25	63.50	8	11.57
9108	B Tx623	B	41.5	31	78.74	8	50.23
9109	915 B	B	56.2	34	86.36	7	49.35
9110	EMS 2447 mut	W	40.7	30	76.20	8	30.46
9111	Tx2737	B	50.9	27	68.58	7	14.58
9112	B Tx623	B	29.9	33	83.82	8	18.14
9113	Tx2737	B	50.8	32	81.28	9	21.09
9114	915 B	B	45.3	36	91.44	8	37.09
9115	EMS 2447 mut	W	47.6	30	76.20	9	33.65
9116	B Tx623	B	43.8	32	81.28	8	29.73
9017	Tx2737 x EMS 2447-1	W	42.0	30	76.20	7	33.72
9017	Tx2737 x EMS 2447-1	B	39.0	33	83.82	8	65.95
9018	Tx2737 x EMS 2447-1	W	41.6	29	73.66	8	38.98
9018	Tx2737 x EMS 2447-1	B	49.4	30	76.20	8	28.03
9019	Tx2737 x EMS 2447-1	W	.	.	.	.	.
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass

9019	Tx2737 x EMS 2447-1	B	47.6	31	78.74	9	55.93
9020	Tx2737 x EMS 2447-1	W	44.5	30	76.20	9	131.42
9020	Tx2737 x EMS 2447-1	B	37.7	32	81.28	9	167.63
9021	Tx2737 x EMS 2447-1	W	.	.	.	.	.
9021	Tx2737 x EMS 2447-1	B	.	.	.	.	.
9022	Tx2737 x EMS 2447-1	W	45.5	35	88.90	8	82.07
9022	Tx2737 x EMS 2447-1	B	39.9	30	76.20	8	23.59
9023	Tx2737 x EMS 2447-1	W	36.9	34	86.36	10	100.27
9023	Tx2737 x EMS 2447-1	B	42.9	38	96.52	9	69.60
9024	Tx2737 x EMS 2447-1	W	33.0	30	76.20	9	20.76
9024	Tx2737 x EMS 2447-1	B	25.6	33	83.82	8	78.85
9025	Tx2737 x EMS 2447-1	W	41.7	21	53.34	11	13.06
9025	Tx2737 x EMS 2447-1	B	34.7	34	86.36	11	74.32
9026	Tx2737 x EMS 2447-1	W	50.5	30	76.20	10	19.87
9026	Tx2737 x EMS 2447-1	B	59.6	32	81.28	10	24.84
9027	Tx2737 x EMS 2447-1	W	53.4	28	71.12	9	71.95
9027	Tx2737 x EMS 2447-1	B	35.4	34	86.36	9	53.52
9028	Tx2737 x EMS 2447-1	W	46.6	29	73.66	8	115.66
9028	Tx2737 x EMS 2447-1	B	40.3	34	86.36	8	61.71
9029	Tx2737 x EMS 2447-1	W	44.1	27	68.58	8	19.04
9029	Tx2737 x EMS 2447-1	B	32.1	38	96.52	10	25.62
9030	Tx2737 x EMS 2447-1	W	23.8	39	99.06	10	55.83
9030	Tx2737 x EMS 2447-1	B	33.9	39	99.06	11	67.38
9031	Tx2737 x EMS 2447-1	W	45.3	30	76.20	8	31.91
9031	Tx2737 x EMS 2447-1	B	68.2	36	91.44	9	90.75
9032	Tx2737 x EMS 2447-1	W	56.8	28	71.12	9	37.60
9032	Tx2737 x EMS 2447-1	B	49.6	34	86.36	10	73.00
9117	Tx2737 x EMS 2447-1	W	46.6	30	76.20	9	26.33
9117	Tx2737 x EMS 2447-1	B	51.3	34	86.36	9	40.57
9118	Tx2737 x EMS 2447-1	W	29.3	26	66.04	10	18.20
9118	Tx2737 x EMS 2447-1	B	36.0	31	78.74	10	29.55
9119	Tx2737 x EMS 2447-1	W	60.4	28	71.12	10	39.41
9119	Tx2737 x EMS 2447-1	B	40.9	32	81.28	9	48.79
9120	Tx2737 x EMS 2447-1	W	46.6	30	76.20	10	40.92
9120	Tx2737 x EMS 2447-1	B	54.1	31	78.74	9	66.92
9121	Tx2737 x EMS 2447-1	W	53.6	24	60.96	10	24.27
9121	Tx2737 x EMS 2447-1	B	50.0	30	76.20	10	62.39
9122	Tx2737 x EMS 2447-1	W	47.9	31	78.74	9	25.36
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9122	Tx2737 x EMS 2447-1	B	67.9	29	73.66	9	51.29
9123	Tx2737 x EMS 2447-1	W	47.3	32	81.28	9	87.93
9123	Tx2737 x EMS 2447-1	B	53.2	31	78.74	9	35.79
9124	Tx2737 x EMS 2447-1	W	49.0	27	68.58	8	17.64
9124	Tx2737 x EMS 2447-1	B	57.5	31	78.74	8	31.07
9125	Tx2737 x EMS 2447-1	W	.	.	.	.	.
9125	Tx2737 x EMS 2447-1	B	68.3	22	55.88	8	26.51
9126	Tx2737 x EMS 2447-1	W	54.7	30	76.20	9	27.54
9126	Tx2737 x EMS 2447-1	B	76.3	27	68.58	8	26.56
9127	Tx2737 x EMS 2447-1	W	.	.	.	.	.
9127	Tx2737 x EMS 2447-1	B	40.3	23	58.42	6	22.55
9128	Tx2737 x EMS 2447-1	W	33.5	25	63.50	8	33.39
9128	Tx2737 x EMS 2447-1	B	46.4	26	66.04	8	39.09
9129	Tx2737 x EMS 2447-1	W	38.3	23	58.42	8	19.24
9129	Tx2737 x EMS 2447-1	B	43.1	25	63.50	9	14.92
9130	Tx2737 x EMS 2447-1	W	47.0	28	71.12	9	31.26
9130	Tx2737 x EMS 2447-1	B	55.8	29	73.66	8	24.53
9131	Tx2737 x EMS 2447-1	W	.	.	.	.	.
9131	Tx2737 x EMS 2447-1	B	35.2	25	63.50	8	66.04
9132	Tx2737 x EMS 2447-1	W	51.1	25	63.50	8	33.69
9132	Tx2737 x EMS 2447-1	B	60.2	21	53.34	8	22.28
9133	Tx2737 x EMS 2447-2	W	45.0	29	73.66	9	29.64
9133	Tx2737 x EMS 2447-2	B	43.8	29	73.66	10	40.83
9134	Tx2737 x EMS 2447-2	W	48.3	31	78.74	10	35.09
9134	Tx2737 x EMS 2447-2	B	37.0	30	76.20	9	25.44
9135	Tx2737 x EMS 2447-2	W	34.0	29	73.66	9	19.53
9135	Tx2737 x EMS 2447-2	B	53.3	31	78.74	10	45.03
9136	Tx2737 x EMS 2447-2	W	52.6	30	76.20	9	61.52
9136	Tx2737 x EMS 2447-2	B	45.9	32	81.28	10	41.68
9137	Tx2737 x EMS 2447-2	W	43.4	27	68.58	8	14.88
9137	Tx2737 x EMS 2447-2	B	66.9	27	68.58	9	25.22
9138	Tx2737 x EMS 2447-2	W	.	.	.	.	.
9138	Tx2737 x EMS 2447-2	B	48.0	29	73.66	9	28.49
9139	Tx2737 x EMS 2447-2	W	43.8	29	73.66	8	27.35
9139	Tx2737 x EMS 2447-2	B	41.4	32	81.28	9	52.62
9140	Tx2737 x EMS 2447-2	W	47.9	30	76.20	9	21.83
9140	Tx2737 x EMS 2447-2	B	50.0	29	73.66	10	35.40
9141	Tx2737 x EMS 2447-2	W	47.9	29	73.66	8	24.94
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9141	Tx2737 x EMS 2447-2	B	54.1	29	73.66	8	35.54
9142	Tx2737 x EMS 2447-2	W	51.2	27	68.58	7	25.85
9142	Tx2737 x EMS 2447-2	B	39.5	27	68.58	9	17.38
9143	Tx2737 x EMS 2447-2	W	33.8	30	76.20	9	51.68
9143	Tx2737 x EMS 2447-2	B	57.5	25	63.50	8	59.93
9144	Tx2737 x EMS 2447-2	W	67.9	25	63.50	8	36.48
9144	Tx2737 x EMS 2447-2	B	56.0	28	71.12	8	38.30
9145	Tx2737 x EMS 2447-2	W	42.1	25	63.50	8	17.98
9145	Tx2737 x EMS 2447-2	B	84.5	27	68.58	9	39.41
9146	Tx2737 x EMS 2447-2	W	40.3	24	60.96	9	26.36
9146	Tx2737 x EMS 2447-2	B	56.3	24	60.96	9	32.83
9147	Tx2737 x EMS 2447-2	W	40.6	24	60.96	8	18.10
9147	Tx2737 x EMS 2447-2	B	52.5	26	66.04	8	29.52
9148	Tx2737 x EMS 2447-2	W	.	.	.	.	.
9148	Tx2737 x EMS 2447-2	B	59.0	25	63.50	9	42.58
9033	Tx2737 x EMS 2447-2	W	47.4	34	86.36	10	50.16
9033	Tx2737 x EMS 2447-2	B	32.3	36	91.44	10	64.06
9034	Tx2737 x EMS 2447-2	W	37.6	31	78.74	8	23.23
9034	Tx2737 x EMS 2447-2	B	49.1	29	73.66	7	12.60
9035	Tx2737 x EMS 2447-2	W	41.8	30	76.20	8	34.90
9035	Tx2737 x EMS 2447-2	B	31.3	27	68.58	8	26.78
9036	Tx2737 x EMS 2447-2	W	33.3	33	83.82	9	61.38
9036	Tx2737 x EMS 2447-2	B	45.1	33	83.82	8	27.79
9037	Tx2737 x EMS 2447-2	W	49.4	38	96.52	8	28.43
9037	Tx2737 x EMS 2447-2	B	48.8	37	93.98	9	40.33
9038	Tx2737 x EMS 2447-2	W	34.7	26	66.04	8	45.16
9038	Tx2737 x EMS 2447-2	B	27.4	38	96.52	10	53.32
9039	Tx2737 x EMS 2447-2	W	43.8	33	83.82	10	67.83
9039	Tx2737 x EMS 2447-2	B	30.7	36	91.44	11	50.15
9040	Tx2737 x EMS 2447-2	W	33.5	36	91.44	8	33.29
9040	Tx2737 x EMS 2447-2	B	32.5	35	88.90	10	56.01
9041	Tx2737 x EMS 2447-2	W	63.6	28	71.12	9	86.89
9041	Tx2737 x EMS 2447-2	B	37.8	39	99.06	10	65.37
9042	Tx2737 x EMS 2447-2	W	46.8	33	83.82	7	20.28
9042	Tx2737 x EMS 2447-2	B	25.7	33	83.82	8	56.89
9043	Tx2737 x EMS 2447-2	W	55.5	34	86.36	9	58.64
9043	Tx2737 x EMS 2447-2	B	45.5	31	78.74	9	55.83
9044	Tx2737 x EMS 2447-2	W	52.0	34	86.36	9	44.82
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9044	Tx2737 x EMS 2447-2	B	42.6	34	86.36	11	136.01
9045	Tx2737 x EMS 2447-2	W	45.2	35	88.90	9	68.60
9045	Tx2737 x EMS 2447-2	B	32.5	35	88.90	12	73.54
9046	Tx2737 x EMS 2447-2	W	32.3	34	86.36	9	36.03
9046	Tx2737 x EMS 2447-2	B	55.0	35	88.90	10	61.84
9047	Tx2737 x EMS 2447-2	W	47.8	31	78.74	8	53.93
9047	Tx2737 x EMS 2447-2	B	35.8	31	78.74	8	45.82
9048	Tx2737 x EMS 2447-2	W	25.9	30	76.20	9	78.33
9048	Tx2737 x EMS 2447-2	B	44.1	34	86.36	8	100.52
9049	EMS 2447 x 915B	W	34.5	25	64	8	17.10
9049	EMS 2447 x 915B	B	44.2	39	99	8	92.86
9050	EMS 2447 x 915B	W	49.1	32	81	7	30.71
9050	EMS 2447 x 915B	B	30.2	37	94	7	36.66
9051	EMS 2447 x 915B	W	52.9	33	84	8	34.75
9051	EMS 2447 x 915B	B	41.5	34	86	8	51.77
9052	EMS 2447 x 915B	W	44.2	38	97	8	112.04
9052	EMS 2447 x 915B	B	39.3	31	79	7	39.80
9053	EMS 2447 x 915B	W	37.7	28	71	7	49.09
9053	EMS 2447 x 915B	B	41.7	33	84	7	45.11
9054	EMS 2447 x 915B	W	40.3	25	64	7	24.06
9054	EMS 2447 x 915B	B	37.9	38	97	8	65.37
9055	EMS 2447 x 915B	W	31.6	32	81	10	39.88
9055	EMS 2447 x 915B	B	31.8	30	76	8	78.42
9056	EMS 2447 x 915B	W	61.5	35	89	8	47.57
9056	EMS 2447 x 915B	B	40.0	30	76	8	30.41
9057	EMS 2447 x 915B	W	49.6	24	61	9	18.05
9057	EMS 2447 x 915B	B	37.9	34	86	8	61.92
9058	EMS 2447 x 915B	W	31.9	27	69	8	25.68
9058	EMS 2447 x 915B	B	38.0	32	81	8	67.28
9059	EMS 2447 x 915B	W	48.2	32	81	8	77.08
9059	EMS 2447 x 915B	B	64.4	33	84	7	86.66
9060	EMS 2447 x 915B	W	30.2	35	89	7	56.57
9060	EMS 2447 x 915B	B	52.7	31	79	8	123.73
9061	EMS 2447 x 915B	W	36.7	30	76	6	13.73
9061	EMS 2447 x 915B	B	37.8	30	76	7	24.19
9062	EMS 2447 x 915B	W	44.2	34	86	7	44.63
9062	EMS 2447 x 915B	B	44.4	36	91	8	57.72
9063	EMS 2447 x 915B	W	33.0	35	89	8	39.60
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9063	EMS 2447 x 915B	B	37.6	44	112	9	54.48
9064	EMS 2447 x 915B	W	41.0	26	66	7	18.97
9064	EMS 2447 x 915B	B	35.2	31	79	8	59.69
9149	EMS 2447 x 915B	W	56.0	38	97	9	48.27
9149	EMS 2447 x 915B	B	56.7	36	91	9	25.21
9150	EMS 2447 x 915B	W	40.7	34	86	8	25.04
9150	EMS 2447 x 915B	B	50.7	34	86	9	37.03
9151	EMS 2447 x 915B	W	40.2	37	94	7	30.50
9151	EMS 2447 x 915B	B	32.5	48	122	10	46.67
9152	EMS 2447 x 915B	W	42.1	36	91	8	42.23
9152	EMS 2447 x 915B	B	42.7	36	91	9	42.92
9153	EMS 2447 x 915B	W	46.4	28	71	8	16.23
9153	EMS 2447 x 915B	B	49.3	37	94	8	50.66
9154	EMS 2447 x 915B	W	55.9	36	91	7	42.10
9154	EMS 2447 x 915B	B	60.5	26	66	7	35.72
9155	EMS 2447 x 915B	W	35.2	39	99	7	22.25
9155	EMS 2447 x 915B	B	42.7	38	97	7	40.76
9156	EMS 2447 x 915B	W	33.7	30	76	8	45.87
9156	EMS 2447 x 915B	B	33.7	39	99	7	25.66
9157	EMS 2447 x 915B	W	48.5	32	81	7	36.42
9157	EMS 2447 x 915B	B	42.0	32	81	7	17.23
9158	EMS 2447 x 915B	W	53.9	27	69	7	31.99
9158	EMS 2447 x 915B	B	48.8	44	112	8	85.37
9159	EMS 2447 x 915B	W	49.2	29	74	8	23.80
9159	EMS 2447 x 915B	B	31.8	36	91	8	22.64
9160	EMS 2447 x 915B	W	44.0	25	64	7	15.65
9160	EMS 2447 x 915B	B	52.1	28	71	7	34.53
9161	EMS 2447 x 915B	W	52.0	30	76	8	40.09
9161	EMS 2447 x 915B	B	47.5	31	79	8	33.55
9162	EMS 2447 x 915B	W	40.1	29	74	7	21.38
9162	EMS 2447 x 915B	B	48.8	30	76	8	34.55
9163	EMS 2447 x 915B	W	62.7	23	58	7	19.10
9163	EMS 2447 x 915B	B	39.1	28	71	7	25.57
9164	EMS 2447 x 915B	W	35.3	34	86	8	32.53
9164	EMS 2447 x 915B	B	47.9	34	86	8	29.10
9065	EMS 2447 x Tx623	W	44.6	33	84	8	43.31
9065	EMS 2447 x Tx623	B	50.2	31	79	7	34.71
9066	EMS 2447 x Tx623	W	41.5	33	84	8	41.89

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass
9066	EMS 2447 x Tx623	B	43.9	29	74	8	21.44
9067	EMS 2447 x Tx623	W	35.7	28	71	8	16.73
9067	EMS 2447 x Tx623	B	25.3	30	76	8	34.08
9068	EMS 2447 x Tx623	W	29.6	24	61	7	11.84
9068	EMS 2447 x Tx623	B	29.2	32	81	8	38.65
9069	EMS 2447 x Tx623	W	36.6	33	84	9	133.85
9069	EMS 2447 x Tx623	B	35.9	33	84	9	45.08
9070	EMS 2447 x Tx623	W	.	.	.	.	.
9070	EMS 2447 x Tx623	B	29.4	32	81	9	43.56
9071	EMS 2447 x Tx623	W	.	.	.	.	.
9071	EMS 2447 x Tx623	B	47.2	32	81	8	44.76
9072	EMS 2447 x Tx623	W	35.9	29	74	7	19.51
9072	EMS 2447 x Tx623	B	33.5	37	94	8	40.23
9073	EMS 2447 x Tx623	W	23.8	37	94	8	19.15
9073	EMS 2447 x Tx623	B	41.6	33	84	8	42.16
9074	EMS 2447 x Tx623	W	28.2	25	64	8	14.81
9074	EMS 2447 x Tx623	B	33.4	31	79	8	41.72
9075	EMS 2447 x Tx623	W	46.0	30	76	8	84.26
9075	EMS 2447 x Tx623	B	40.8	29	74	8	82.63
9076	EMS 2447 x Tx623	W	50.7	30	76	8	30.98
9076	EMS 2447 x Tx623	B	39.6	29	74	9	44.58
9077	EMS 2447 x Tx623	W	30.6	31	79	8	10.29
9077	EMS 2447 x Tx623	B	33.2	35	89	8	29.90
9078	EMS 2447 x Tx623	W	43.0	32	81	9	50.01
9078	EMS 2447 x Tx623	B	37.8	32	81	8	56.23
9079	EMS 2447 x Tx623	W	41.6	33	84	8	37.89
9079	EMS 2447 x Tx623	B	52.3	37	94	9	53.00
9080	EMS 2447 x Tx623	W	42.8	30	76	7	18.69
9080	EMS 2447 x Tx623	B	38.1	31	79	7	37.29
9165	EMS 2447 x Tx623	W	51.5	26	66	9	37.32
9165	EMS 2447 x Tx623	B	44.7	33	84	8	28.48
9166	EMS 2447 x Tx623	W	43.3	33	84	10	58.53
9166	EMS 2447 x Tx623	B	5.0	28	71	10	46.83
9167	EMS 2447 x Tx623	W	68.1	25	64	9	60.05
9167	EMS 2447 x Tx623	B	58.5	30	76	9	75.62
9168	EMS 2447 x Tx623	W	41.9	31	79	9	62.48
9168	EMS 2447 x Tx623	B	35.6	33	84	8	42.19
9169	EMS 2447 x Tx623	W	63.5	22	56	7	23.78
Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Leaf #	Biomass

9169	EMS 2447 x Tx623	B	46.5	34	86	10	63.64
9170	EMS 2447 x Tx623	W	.	.	.	.	.
9170	EMS 2447 x Tx623	B	31.3	34	86	9	31.00
9171	EMS 2447 x Tx623	W	53.8	35	89	9	50.64
9171	EMS 2447 x Tx623	B	43.7	39	99	8	41.58
9172	EMS 2447 x Tx623	W	50.2	32	81	8	35.11
9172	EMS 2447 x Tx623	B	37.4	31	79	9	41.67
9173	EMS 2447 x Tx623	W	58.6	23	58	7	27.82
9173	EMS 2447 x Tx623	B	42.7	29	74	8	34.49
9174	EMS 2447 x Tx623	W	59.5	28	71	8	75.04
9174	EMS 2447 x Tx623	B	35.0	32	81	7	30.44
9175	EMS 2447 x Tx623	W	58.3	28	71	8	38.77
9175	EMS 2447 x Tx623	B	56.6	33	84	9	34.58
9176	EMS 2447 x Tx623	W	40.7	28	71	8	50.50
9176	EMS 2447 x Tx623	B	41.4	29	74	8	52.06
9177	EMS 2447 x Tx623	W	31.1	31	79	9	47.17
9177	EMS 2447 x Tx623	B	46.6	30	76	9	41.63
9178	EMS 2447 x Tx623	W	46.8	29	74	9	47.27
9178	EMS 2447 x Tx623	B	38.6	25	64	8	22.21
9179	EMS 2447 x Tx623	W	59.2	24	61	8	22.69
9179	EMS 2447 x Tx623	B	48.3	28	71	8	40.06
9180	EMS 2447 x Tx623	W	45.3	29	74	9	56.10
9180	EMS 2447 x Tx623	B	53.4	22	56	7	37.12

3. <u>Growth and Development Experiment Week 12 Sampling Data</u>

Plot	Pedigree	HCN Test	Chlorophyll
9101	B Tx623	B	37.9
9102	915 B	B	55.6
9103	Tx2737	B	56.9
9104	EMS 2447 mut	W	46.3
9105	915 B	B	16.9
9106	Tx2737	B	43.8
9107	EMS 2447 mut	W	50.0
9108	B Tx623	B	48.4
9109	915 B	B	56.7
9110	EMS 2447 mut	W	40.0
9111	Tx2737	B	60.3
9112	B Tx623	B	34.7
9113	Tx2737	B	53.5
9114	915 B	B	59.5
9115	EMS 2447 mut	W	20.7
9116	B Tx623	B	65.6
9001	EMS 2447 mut	W	47.8
9002	Tx2737	B	86.2
9003	B Tx623	B	42.7
9004	915 B	B	53.3
9005	915 B	B	48.9
9006	EMS 2447 mut	W	47.0
9007	B Tx623	B	58.9
9008	Tx2737	B	80.6
9009	B Tx623	B	42.7
9010	915 B	B	49.5
9011	EMS 2447 mut	W	35.9
9012	Tx2737	B	75.9
9013	EMS 2447 mut	W	56.0
9014	B Tx623	B	53.3
9015	Tx2737	B	76.1
9016	915 B	B	48.9
9117	Tx2737 x EMS 2447-1	W	72.1
9118	Tx2737 x EMS 2447-1	W	69.4
9119	Tx2737 x EMS 2447-1	W	.
9120	Tx2737 x EMS 2447-1	W	71.2

Plot	Pedigree	HCN Test	Chlorophyll
9121	Tx2737 x EMS 2447-1	W	61.6
9122	Tx2737 x EMS 2447-1	W	.
9123	Tx2737 x EMS 2447-1	W	62.2
9124	Tx2737 x EMS 2447-1	W	74.4
9125	Tx2737 x EMS 2447-1	W	.
9126	Tx2737 x EMS 2447-1	W	69.3
9127	Tx2737 x EMS 2447-1	W	.
9128	Tx2737 x EMS 2447-1	W	.
9129	Tx2737 x EMS 2447-1	W	70.0
9130	Tx2737 x EMS 2447-1	W	66.5
9131	Tx2737 x EMS 2447-1	W	.
9132	Tx2737 x EMS 2447-1	W	64.3
9117	Tx2737 x EMS 2447-1	B	54.4
9118	Tx2737 x EMS 2447-1	B	71.2
9119	Tx2737 x EMS 2447-1	B	63.0
9120	Tx2737 x EMS 2447-1	B	46.9
9121	Tx2737 x EMS 2447-1	B	59.9
9122	Tx2737 x EMS 2447-1	B	62.9
9123	Tx2737 x EMS 2447-1	B	59.4
9124	Tx2737 x EMS 2447-1	B	70.3
9125	Tx2737 x EMS 2447-1	B	52.0
9126	Tx2737 x EMS 2447-1	B	73.8
9127	Tx2737 x EMS 2447-1	B	53.2
9128	Tx2737 x EMS 2447-1	B	72.3
9129	Tx2737 x EMS 2447-1	B	80.7
9130	Tx2737 x EMS 2447-1	B	73.9
9131	Tx2737 x EMS 2447-1	B	.
9132	Tx2737 x EMS 2447-1	B	68.3
9017	Tx2737 x EMS 2447-1	W	.
9018	Tx2737 x EMS 2447-1	W	33.4
9019	Tx2737 x EMS 2447-1	W	.
9020	Tx2737 x EMS 2447-1	W	63.9
9021	Tx2737 x EMS 2447-1	W	.
9022	Tx2737 x EMS 2447-1	W	83.5
9023	Tx2737 x EMS 2447-1	W	61.0
9024	Tx2737 x EMS 2447-1	W	66.9
9025	Tx2737 x EMS 2447-1	W	29.0

Plot	Pedigree	HCN Test	Chlorophyll
9026	Tx2737 x EMS 2447-1	W	47.4
9027	Tx2737 x EMS 2447-1	W	71.9
9028	Tx2737 x EMS 2447-1	W	61.1
9029	Tx2737 x EMS 2447-1	W	45.1
9030	Tx2737 x EMS 2447-1	W	41.1
9031	Tx2737 x EMS 2447-1	W	61.0
9032	Tx2737 x EMS 2447-1	W	75.5
9017	Tx2737 x EMS 2447-1	B	58.9
9018	Tx2737 x EMS 2447-1	B	40.7
9019	Tx2737 x EMS 2447-1	B	59.4
9020	Tx2737 x EMS 2447-1	B	59.9
9021	Tx2737 x EMS 2447-1	B	43.9
9022	Tx2737 x EMS 2447-1	B	63.9
9023	Tx2737 x EMS 2447-1	B	63.1
9024	Tx2737 x EMS 2447-1	B	62.0
9025	Tx2737 x EMS 2447-1	B	81.3
9026	Tx2737 x EMS 2447-1	B	47.8
9027	Tx2737 x EMS 2447-1	B	69.3
9028	Tx2737 x EMS 2447-1	B	48.9
9029	Tx2737 x EMS 2447-1	B	72.3
9030	Tx2737 x EMS 2447-1	B	66.7
9031	Tx2737 x EMS 2447-1	B	36.9
9032	Tx2737 x EMS 2447-1	B	59.0
9133	Tx2737 x EMS 2447-2	W	37.9
9134	Tx2737 x EMS 2447-2	W	53.0
9135	Tx2737 x EMS 2447-2	W	43.4
9136	Tx2737 x EMS 2447-2	W	69.9
9137	Tx2737 x EMS 2447-2	W	58.5
9138	Tx2737 x EMS 2447-2	W	.
9139	Tx2737 x EMS 2447-2	W	60.7
9140	Tx2737 x EMS 2447-2	W	55.0
9141	Tx2737 x EMS 2447-2	W	60.0
9142	Tx2737 x EMS 2447-2	W	76.2
9143	Tx2737 x EMS 2447-2	W	44.1
9144	Tx2737 x EMS 2447-2	W	62.9
9145	Tx2737 x EMS 2447-2	W	53.8
9146	Tx2737 x EMS 2447-2	W	82.5

Plot	Pedigree	HCN Test	Chlorophyll
9147	Tx2737 x EMS 2447-2	W	59.4
9148	Tx2737 x EMS 2447-2	W	.
9133	Tx2737 x EMS 2447-2	B	77.8
9134	Tx2737 x EMS 2447-2	B	83.3
9135	Tx2737 x EMS 2447-2	B	60.3
9136	Tx2737 x EMS 2447-2	B	54.6
9137	Tx2737 x EMS 2447-2	B	59.9
9138	Tx2737 x EMS 2447-2	B	34.9
9139	Tx2737 x EMS 2447-2	B	51.2
9140	Tx2737 x EMS 2447-2	B	63.9
9141	Tx2737 x EMS 2447-2	B	42.1
9142	Tx2737 x EMS 2447-2	B	73.6
9143	Tx2737 x EMS 2447-2	B	82.2
9144	Tx2737 x EMS 2447-2	B	67.9
9145	Tx2737 x EMS 2447-2	B	52.3
9146	Tx2737 x EMS 2447-2	B	63.7
9147	Tx2737 x EMS 2447-2	B	65.9
9148	Tx2737 x EMS 2447-2	B	61.5
9033	Tx2737 x EMS 2447-2	W	67.3
9034	Tx2737 x EMS 2447-2	W	60.7
9035	Tx2737 x EMS 2447-2	W	71.0
9036	Tx2737 x EMS 2447-2	W	54.0
9037	Tx2737 x EMS 2447-2	W	61.3
9038	Tx2737 x EMS 2447-2	W	45.6
9039	Tx2737 x EMS 2447-2	W	71.4
9040	Tx2737 x EMS 2447-2	W	51.0
9041	Tx2737 x EMS 2447-2	W	57.8
9042	Tx2737 x EMS 2447-2	W	65.2
9043	Tx2737 x EMS 2447-2	W	63.0
9044	Tx2737 x EMS 2447-2	W	63.8
9045	Tx2737 x EMS 2447-2	W	57.7
9046	Tx2737 x EMS 2447-2	W	63.6
9047	Tx2737 x EMS 2447-2	W	52.9
9048	Tx2737 x EMS 2447-2	W	.
9033	Tx2737 x EMS 2447-2	B	56.0
9034	Tx2737 x EMS 2447-2	B	48.7
9035	Tx2737 x EMS 2447-2	B	55.6

Plot	Pedigree	HCN Test	Chlorophyll
9036	Tx2737 x EMS 2447-2	B	58.7
9037	Tx2737 x EMS 2447-2	B	77.2
9038	Tx2737 x EMS 2447-2	B	71.8
9039	Tx2737 x EMS 2447-2	B	47.4
9040	Tx2737 x EMS 2447-2	B	60.3
9041	Tx2737 x EMS 2447-2	B	55.4
9042	Tx2737 x EMS 2447-2	B	76.1
9043	Tx2737 x EMS 2447-2	B	60.8
9044	Tx2737 x EMS 2447-2	B	67.0
9045	Tx2737 x EMS 2447-2	B	50.7
9046	Tx2737 x EMS 2447-2	B	66.0
9047	Tx2737 x EMS 2447-2	B	48.2
9048	Tx2737 x EMS 2447-2	B	74.4
9049	EMS 2447 x 915B	W	50.9
9050	EMS 2447 x 915B	W	48.8
9051	EMS 2447 x 915B	W	.
9052	EMS 2447 x 915B	W	51.4
9053	EMS 2447 x 915B	W	47.4
9054	EMS 2447 x 915B	W	56.2
9055	EMS 2447 x 915B	W	37.8
9056	EMS 2447 x 915B	W	47.9
9057	EMS 2447 x 915B	W	45.2
9058	EMS 2447 x 915B	W	38.1
9059	EMS 2447 x 915B	W	46.3
9060	EMS 2447 x 915B	W	51.3
9061	EMS 2447 x 915B	W	61.7
9062	EMS 2447 x 915B	W	51.7
9063	EMS 2447 x 915B	W	21.5
9064	EMS 2447 x 915B	W	40.5
9049	EMS 2447 x 915B	B	57.9
9050	EMS 2447 x 915B	B	41.9
9051	EMS 2447 x 915B	B	55.7
9052	EMS 2447 x 915B	B	59.1
9053	EMS 2447 x 915B	B	65.3
9054	EMS 2447 x 915B	B	32.4
9055	EMS 2447 x 915B	B	44.8
9056	EMS 2447 x 915B	B	53.7

Plot	Pedigree	HCN Test	Chlorophyll
9057	EMS 2447 x 915B	B	37.2
9058	EMS 2447 x 915B	B	37.6
9059	EMS 2447 x 915B	B	51.9
9060	EMS 2447 x 915B	B	58.7
9061	EMS 2447 x 915B	B	60.8
9062	EMS 2447 x 915B	B	41.5
9063	EMS 2447 x 915B	B	45.9
9064	EMS 2447 x 915B	B	55.1
9149	EMS 2447 x 915B	W	39.9
9150	EMS 2447 x 915B	W	40.5
9151	EMS 2447 x 915B	W	52.1
9152	EMS 2447 x 915B	W	54.8
9153	EMS 2447 x 915B	W	49.1
9154	EMS 2447 x 915B	W	48.6
9155	EMS 2447 x 915B	W	59.6
9156	EMS 2447 x 915B	W	43.9
9157	EMS 2447 x 915B	W	.
9158	EMS 2447 x 915B	W	56.5
9159	EMS 2447 x 915B	W	60.1
9160	EMS 2447 x 915B	W	40.7
9161	EMS 2447 x 915B	W	.
9162	EMS 2447 x 915B	W	50.0
9163	EMS 2447 x 915B	W	54.7
9164	EMS 2447 x 915B	W	63.8
9149	EMS 2447 x 915B	B	48.2
9150	EMS 2447 x 915B	B	54.8
9151	EMS 2447 x 915B	B	45.3
9152	EMS 2447 x 915B	B	38.1
9153	EMS 2447 x 915B	B	41.3
9154	EMS 2447 x 915B	B	50.7
9155	EMS 2447 x 915B	B	51.8
9156	EMS 2447 x 915B	B	53.6
9157	EMS 2447 x 915B	B	47.9
9158	EMS 2447 x 915B	B	57.9
9159	EMS 2447 x 915B	B	48.1
9160	EMS 2447 x 915B	B	55.7
9161	EMS 2447 x 915B	B	48.3

Plot	Pedigree	HCN Test	Chlorophyll
9162	EMS 2447 x 915B	B	48.3
9163	EMS 2447 x 915B	B	40.2
9164	EMS 2447 x 915B	B	62.5
9065	EMS 2447 x Tx623	W	.
9066	EMS 2447 x Tx623	W	29.2
9067	EMS 2447 x Tx623	W	.
9068	EMS 2447 x Tx623	W	50.2
9069	EMS 2447 x Tx623	W	23.8
9070	EMS 2447 x Tx623	W	.
9071	EMS 2447 x Tx623	W	.
9072	EMS 2447 x Tx623	W	36.8
9073	EMS 2447 x Tx623	W	47.4
9074	EMS 2447 x Tx623	W	51.8
9075	EMS 2447 x Tx623	W	56.9
9076	EMS 2447 x Tx623	W	64.7
9077	EMS 2447 x Tx623	W	53.7
9078	EMS 2447 x Tx623	W	.
9079	EMS 2447 x Tx623	W	.
9080	EMS 2447 x Tx623	W	.
9065	EMS 2447 x Tx623	B	64.2
9066	EMS 2447 x Tx623	B	45.4
9067	EMS 2447 x Tx623	B	57.7
9068	EMS 2447 x Tx623	B	55.9
9069	EMS 2447 x Tx623	B	73.7
9070	EMS 2447 x Tx623	B	42.8
9071	EMS 2447 x Tx623	B	41.0
9072	EMS 2447 x Tx623	B	51.9
9073	EMS 2447 x Tx623	B	59.2
9074	EMS 2447 x Tx623	B	45.4
9075	EMS 2447 x Tx623	B	61.9
9076	EMS 2447 x Tx623	B	58.0
9077	EMS 2447 x Tx623	B	44.2
9078	EMS 2447 x Tx623	B	61.9
9079	EMS 2447 x Tx623	B	66.4
9080	EMS 2447 x Tx623	B	71.9
9165	EMS 2447 x Tx623	W	.
9166	EMS 2447 x Tx623	W	.

Plot	Pedigree	HCN Test	Chlorophyll
9167	EMS 2447 x Tx623	W	58.4
9168	EMS 2447 x Tx623	W	.
9169	EMS 2447 x Tx623	W	45.8
9170	EMS 2447 x Tx623	W	.
9171	EMS 2447 x Tx623	W	37.6
9172	EMS 2447 x Tx623	W	51.4
9173	EMS 2447 x Tx623	W	54.9
9174	EMS 2447 x Tx623	W	.
9175	EMS 2447 x Tx623	W	45.9
9176	EMS 2447 x Tx623	W	.
9177	EMS 2447 x Tx623	W	52.5
9178	EMS 2447 x Tx623	W	41.1
9179	EMS 2447 x Tx623	W	19.9
9180	EMS 2447 x Tx623	W	38.5
9165	EMS 2447 x Tx623	B	32.5
9166	EMS 2447 x Tx623	B	50.6
9167	EMS 2447 x Tx623	B	50.5
9168	EMS 2447 x Tx623	B	42.6
9169	EMS 2447 x Tx623	B	31.8
9170	EMS 2447 x Tx623	B	40.4
9171	EMS 2447 x Tx623	B	67.1
9172	EMS 2447 x Tx623	B	57.3
9173	EMS 2447 x Tx623	B	55.3
9174	EMS 2447 x Tx623	B	47.9
9175	EMS 2447 x Tx623	B	52.3
9176	EMS 2447 x Tx623	B	46.8
9177	EMS 2447 x Tx623	B	44.0
9178	EMS 2447 x Tx623	B	63.6
9179	EMS 2447 x Tx623	B	25.8
9180	EMS 2447 x Tx623	B	41.8

4. Growth and Development Experiment Week 17 (Harvest) Sampling Data

4.1 Chlorophyll Content Index, Plant Height, and Grain Yield

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9001	EMS 2447 mut	W	19.2	33	84	30.80
9002	Tx2737	B	61.9	44	112	90.34
9003	B Tx623	B	27.6	51	130	108.38
9004	915 B	B	37.6	69	175	81.29
9005	915 B	B	43.4	74	188	73.63
9006	EMS 2447 mut	W	17.8	39	99	19.36
9007	B Tx623	B	30.6	59	150	82.12
9008	Tx2737	B	68.1	43	109	68.58
9009	B Tx623	B	42.1	53	135	111.48
9010	915 B	B	29.3	73	185	68.93
9011	EMS 2447 mut	W	16.2	43	109	34.69
9012	Tx2737	B	84.7	47	119	57.67
9013	EMS 2447 mut	W	29.9	39	99	39.68
9014	B Tx623	B	27.9	67	170	64.73
9015	Tx2737	B	33.9	50	127	43.41
9016	915 B	B	47.8	75	191	75.14
9101	B Tx623	B	22.1	66	168	47.51
9102	915 B	B	37.1	75	191	74.53
9103	Tx2737	B	40.3	48	122	35.95
9104	EMS 2447 mut	W	59.2	47	119	23.14
9105	915 B	B	32.3	77	196	68.66
9106	Tx2737	B	76.5	49	124	58.12
9107	EMS 2447 mut	W	33.5	40	102	15.94
9108	B Tx623	B	36.8	62	157	100.94
9109	915 B	B	54.1	72	183	104.65
9110	EMS 2447 mut	W	36.0	48	122	45.18
9112	B Tx623	B	35.8	73	185	69.94
9113	Tx2737	B	56.0	49	124	33.84
9114	915 B	B	31.2	77	196	83.97
9115	EMS 2447 mut	W	35.9	40	102	46.56
9116	B Tx623	B	31.40	65	165	93.10
9017	Tx2737 x EMS 2447-1	W	.	.	.	.
9018	Tx2737 x EMS 2447-1	W	.	.	.	.

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9019	Tx2737 x EMS 2447-1	W	.	.	.	.
9020	Tx2737 x EMS 2447-1	W	.	.	.	.
9021	Tx2737 x EMS 2447-1	W	.	.	.	.
9022	Tx2737 x EMS 2447-1	W	48.8	55	140	55.62
9023	Tx2737 x EMS 2447-1	W	48.2	48	122	40.79
9024	Tx2737 x EMS 2447-1	W	26.9	46	117	44.78
9025	Tx2737 x EMS 2447-1	W	72.3	42	107	42.95
9026	Tx2737 x EMS 2447-1	W	22.3	55	140	79.72
9027	Tx2737 x EMS 2447-1	W	48.9	47	119	82.84
9028	Tx2737 x EMS 2447-1	W	26.7	49	124	50.72
9029	Tx2737 x EMS 2447-1	W	30.2	51	130	47.62
9030	Tx2737 x EMS 2447-1	W	35.0	53	135	68.35
9031	Tx2737 x EMS 2447-1	W	34.0	46	117	43.23
9032	Tx2737 x EMS 2447-1	W	62.6	45	114	44.17
9017	Tx2737 x EMS 2447-1	B	31.3	48	122	127.75
9018	Tx2737 x EMS 2447-1	B	32.3	49	124	112.77
9019	Tx2737 x EMS 2447-1	B	68.0	42	107	66.50
9020	Tx2737 x EMS 2447-1	B	42.6	44	112	125.64
9021	Tx2737 x EMS 2447-1	B	38.3	57	145	34.28
9022	Tx2737 x EMS 2447-1	B	49.4	52	132	85.22
9023	Tx2737 x EMS 2447-1	B	64.3	49	124	118.01
9024	Tx2737 x EMS 2447-1	B	40.5	51	130	86.98
9025	Tx2737 x EMS 2447-1	B	16.4	42	107	53.72
9026	Tx2737 x EMS 2447-1	B	56.5	51	130	120.26
9027	Tx2737 x EMS 2447-1	B	32.3	50	127	77.45
9028	Tx2737 x EMS 2447-1	B	27.3	62	157	56.06
9029	Tx2737 x EMS 2447-1	B	33.8	51	130	66.31
9030	Tx2737 x EMS 2447-1	B	33.4	53	135	79.96
9031	Tx2737 x EMS 2447-1	B	37.5	50	127	51.96
9032	Tx2737 x EMS 2447-1	B	39.2	55	140	60.92
9117	Tx2737 x EMS 2447-1	B	55.4	58	147	79.27
9118	Tx2737 x EMS 2447-1	B	46.0	57	145	111.84
9119	Tx2737 x EMS 2447-1	B	47.5	50	127	108.47
9120	Tx2737 x EMS 2447-1	B	60.0	44	112	27.25
9121	Tx2737 x EMS 2447-1	B	40.2	55	140	90.28
9122	Tx2737 x EMS 2447-1	B	55.9	54	137	109.07
9123	Tx2737 x EMS 2447-1	B	54.1	43	109	101.99
9124	Tx2737 x EMS 2447-1	B	46.2	48	122	58.33

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9125	Tx2737 x EMS 2447-1	B	11.2	44	112	95.07
9126	Tx2737 x EMS 2447-1	B	39.4	50	127	64.86
9127	Tx2737 x EMS 2447-1	B	63.0	45	114	80.07
9128	Tx2737 x EMS 2447-1	B	56.2	47	119	119.45
9129	Tx2737 x EMS 2447-1	B	60.9	57	145	71.25
9130	Tx2737 x EMS 2447-1	B	58.7	47	119	56.80
9131	Tx2737 x EMS 2447-1	B	.	.	.	.
9132	Tx2737 x EMS 2447-1	B	61.5	51	130	83.12
9117	Tx2737 x EMS 2447-1	W	71.7	46	117	.
9118	Tx2737 x EMS 2447-1	W	66.6	49	124	47.23
9119	Tx2737 x EMS 2447-1	W	.	.	.	.
9120	Tx2737 x EMS 2447-1	W	58.0	42	107	5.00
9121	Tx2737 x EMS 2447-1	W	60.7	53	135	44.42
9122	Tx2737 x EMS 2447-1	W	.	.	.	.
9123	Tx2737 x EMS 2447-1	W	52.6	48	122	49.58
9124	Tx2737 x EMS 2447-1	W	37.5	49	124	71.07
9125	Tx2737 x EMS 2447-1	W	.	.	.	.
9126	Tx2737 x EMS 2447-1	W	58.8	49	124	30.25
9127	Tx2737 x EMS 2447-1	W	.	.	.	.
9128	Tx2737 x EMS 2447-1	W	.	.	.	.
9129	Tx2737 x EMS 2447-1	W	53.9	61	155	51.30
9130	Tx2737 x EMS 2447-1	W	44.7	54	137	.
9131	Tx2737 x EMS 2447-1	W	.	.	.	.
9132	Tx2737 x EMS 2447-1	W	48.0	52	132	76.51
9033	Tx2737 x EMS 2447-2	W	50.3	42	107	43.63
9034	Tx2737 x EMS 2447-2	W	25.3	44	112	23.35
9035	Tx2737 x EMS 2447-2	W	46.3	45	114	70.47
9036	Tx2737 x EMS 2447-2	W	31.5	45	114	52.21
9037	Tx2737 x EMS 2447-2	W	37.4	49	124	49.38
9038	Tx2737 x EMS 2447-2	W	44.1	50	127	38.88
9039	Tx2737 x EMS 2447-2	W	40.3	48	122	31.57
9040	Tx2737 x EMS 2447-2	W	52.1	50	127	30.10
9041	Tx2737 x EMS 2447-2	W	43.3	50	127	58.00
9042	Tx2737 x EMS 2447-2	W	39.5	48	122	28.24
9043	Tx2737 x EMS 2447-2	W	39.0	52	132	41.43
9044	Tx2737 x EMS 2447-2	W	45.7	51	130	56.33
9045	Tx2737 x EMS 2447-2	W	39.4	51	130	62.94
9046	Tx2737 x EMS 2447-2	W	47.3	51	130	41.82

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9047	Tx2737 x EMS 2447-2	W	26.5	51	130	48.86
9048	Tx2737 x EMS 2447-2	W	.	.	.	.
9033	Tx2737 x EMS 2447-2	B	35.7	49	124	67.05
9034	Tx2737 x EMS 2447-2	B	32.2	49	124	80.93
9035	Tx2737 x EMS 2447-2	B	31.0	56	142	51.38
9036	Tx2737 x EMS 2447-2	B	32.8	53	135	68.79
9037	Tx2737 x EMS 2447-2	B	62.6	49	124	85.61
9038	Tx2737 x EMS 2447-2	B	47.1	55	140	71.46
9039	Tx2737 x EMS 2447-2	B	56.4	55	140	51.94
9040	Tx2737 x EMS 2447-2	B	37.4	54	137	93.09
9041	Tx2737 x EMS 2447-2	B	40.8	53	135	51.23
9042	Tx2737 x EMS 2447-2	B	37.3	48	122	132.31
9043	Tx2737 x EMS 2447-2	B	39.8	52	132	88.11
9044	Tx2737 x EMS 2447-2	B	39.9	55	140	53.48
9045	Tx2737 x EMS 2447-2	B	67.6	47	119	52.96
9046	Tx2737 x EMS 2447-2	B	31.4	50	127	78.47
9047	Tx2737 x EMS 2447-2	B	43.5	56	142	40.29
9048	Tx2737 x EMS 2447-2	B	18.6	45	114	101.37
9133	Tx2737 x EMS 2447-2	B	37.30	59	150	100.23
9134	Tx2737 x EMS 2447-2	B	56.50	48	122	76.77
9135	Tx2737 x EMS 2447-2	B	53.70	54	137	127.20
9136	Tx2737 x EMS 2447-2	B	47.20	58	147	88.68
9137	Tx2737 x EMS 2447-2	B	55.20	52	132	61.71
9138	Tx2737 x EMS 2447-2	B	50.10	51	130	83.82
9139	Tx2737 x EMS 2447-2	B	48.30	62	157	54.83
9140	Tx2737 x EMS 2447-2	B	74.90	47	119	69.75
9141	Tx2737 x EMS 2447-2	B	25.20	61	155	69.73
9142	Tx2737 x EMS 2447-2	B	52.00	49	124	100.13
9143	Tx2737 x EMS 2447-2	B	47.50	54	137	73.51
9144	Tx2737 x EMS 2447-2	B	47.30	51	130	46.92
9145	Tx2737 x EMS 2447-2	B	45.00	58	147	74.82
9146	Tx2737 x EMS 2447-2	B	42.20	56	142	64.70
9147	Tx2737 x EMS 2447-2	B	69.10	53	135	54.96
9148	Tx2737 x EMS 2447-2	B	50.00	47	119	75.51
9133	Tx2737 x EMS 2447-2	B	27.40	54	137	47.22
9134	Tx2737 x EMS 2447-2	W	63.60	52	132	68.88
9135	Tx2737 x EMS 2447-2	W	32.20	68	173	.
9136	Tx2737 x EMS 2447-2	W	58.20	55	140	64.31

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9137	Tx2737 x EMS 2447-2	W	43.90	50	127	72.49
9138	Tx2737 x EMS 2447-2	W	.	.	.	.
9139	Tx2737 x EMS 2447-2	W	23.20	58	147	45.46
9140	Tx2737 x EMS 2447-2	W	41.30	59	150	49.50
9141	Tx2737 x EMS 2447-2	W	50.50	50	127	41.63
9142	Tx2737 x EMS 2447-2	W	16.30	49	124	51.92
9143	Tx2737 x EMS 2447-2	W	34.90	55	140	68.16
9144	Tx2737 x EMS 2447-2	W	42.90	48	122	55.96
9145	Tx2737 x EMS 2447-2	W	47.80	50	127	24.08
9146	Tx2737 x EMS 2447-2	W	34.60	55	140	56.97
9147	Tx2737 x EMS 2447-2	W	64.20	54	137	36.12
9148	Tx2737 x EMS 2447-2	W	.	.	.	.
9149	EMS 2447 x 915B	B	36.50	77	196	96.74
9150	EMS 2447 x 915B	B	37.10	80	203	44.06
9151	EMS 2447 x 915B	B	52.00	76	193	107.82
9152	EMS 2447 x 915B	B	19.90	85	216	23.36
9153	EMS 2447 x 915B	B	36.90	74	188	111.06
9154	EMS 2447 x 915B	B	37.50	85	216	10.26
9155	EMS 2447 x 915B	B	20.00	93	236	60.73
9156	EMS 2447 x 915B	B	44.50	75	191	90.22
9157	EMS 2447 x 915B	B	24.00	74	188	47.20
9158	EMS 2447 x 915B	B	34.80	72	183	78.06
9159	EMS 2447 x 915B	B	43.60	86	218	95.78
9160	EMS 2447 x 915B	B	45.80	84	213	79.75
9161	EMS 2447 x 915B	B	55.70	81	206	101.91
9162	EMS 2447 x 915B	B	34.20	81	206	46.22
9163	EMS 2447 x 915B	B	44.40	78	198	101.45
9164	EMS 2447 x 915B	B	45.00	76	193	101.08
9149	EMS 2447 x 915B	W	24.50	76	193	89.41
9150	EMS 2447 x 915B	W	48.90	66	168	151.00
9151	EMS 2447 x 915B	W	34.60	93	236	.
9152	EMS 2447 x 915B	W	25.00	79	201	.
9153	EMS 2447 x 915B	W	25.40	84	213	59.60
9154	EMS 2447 x 915B	W	32.80	82	208	92.17
9155	EMS 2447 x 915B	W	37.60	89	226	119.29
9156	EMS 2447 x 915B	W	28.20	75	191	33.05
9157	EMS 2447 x 915B	W	.	.	.	.
9158	EMS 2447 x 915B	W	25.20	74	188	62.88

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9159	EMS 2447 x 915B	W	42.90	78	198	74.75
9160	EMS 2447 x 915B	W	31.10	83	211	.
9161	EMS 2447 x 915B	W	.	.	.	.
9162	EMS 2447 x 915B	W	41.90	68	173	.
9163	EMS 2447 x 915B	W	33.00	78	198	67.80
9164	EMS 2447 x 915B	W	55.10	74	188	49.97
9049	EMS 2447 x 915B	W	37.1	57	145	61.72
9050	EMS 2447 x 915B	W	35.2	47	119	48.95
9051	EMS 2447 x 915B	W	.	.	.	.
9052	EMS 2447 x 915B	W	26.1	80	203	72.11
9053	EMS 2447 x 915B	W	47.5	54	137	27.64
9054	EMS 2447 x 915B	W	27.2	71	180	52.33
9055	EMS 2447 x 915B	W	34.4	76	193	9.57
9056	EMS 2447 x 915B	W	36.4	56	142	25.72
9057	EMS 2447 x 915B	W	32.3	77	196	52.83
9058	EMS 2447 x 915B	W	24.3	90	229	55.89
9059	EMS 2447 x 915B	W	40.1	77	196	.
9060	EMS 2447 x 915B	W	30.9	76	193	38.41
9061	EMS 2447 x 915B	W	55.7	62	157	72.12
9062	EMS 2447 x 915B	W	42.4	79	201	62.28
9063	EMS 2447 x 915B	W	27.1	81	206	83.71
9064	EMS 2447 x 915B	W	25.5	86	218	43.46
9049	EMS 2447 x 915B	B	38.0	60	152	91.14
9050	EMS 2447 x 915B	B	10.0	75	191	90.59
9051	EMS 2447 x 915B	B	29.1	76	193	60.71
9052	EMS 2447 x 915B	B	55.8	67	170	130.63
9053	EMS 2447 x 915B	B	47.6	60	152	57.03
9054	EMS 2447 x 915B	B	43.1	71	180	52.21
9055	EMS 2447 x 915B	B	29.2	76	193	76.24
9056	EMS 2447 x 915B	B	36.0	81	206	75.85
9057	EMS 2447 x 915B	B	57.3	71	180	93.99
9058	EMS 2447 x 915B	B	33.2	95	241	118.88
9059	EMS 2447 x 915B	B	46.0	73	185	87.60
9060	EMS 2447 x 915B	B	18.5	78	198	54.42
9061	EMS 2447 x 915B	B	46.4	80	203	103.40
9062	EMS 2447 x 915B	B	20.1	74	188	123.19
9063	EMS 2447 x 915B	B	40.1	77	196	110.52
9064	EMS 2447 x 915B	B	26.3	86	218	44.50

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9065	EMS 2447 x Tx623	W	.	.	.	.
9066	EMS 2447 x Tx623	W	6.7	42	107	49.54
9067	EMS 2447 x Tx623	W	.	.	.	.
9068	EMS 2447 x Tx623	W	10.2	38	97	13.32
9069	EMS 2447 x Tx623	W	12.5	55	140	51.16
9070	EMS 2447 x Tx623	W	.	.	.	.
9071	EMS 2447 x Tx623	W	.	.	.	.
9072	EMS 2447 x Tx623	W	16.2	40	102	19.13
9073	EMS 2447 x Tx623	W	25.2	60	152	61.39
9074	EMS 2447 x Tx623	W	62.9	46	117	95.06
9075	EMS 2447 x Tx623	W	35.0	46	117	55.33
9076	EMS 2447 x Tx623	W	28.3	40	102	29.88
9077	EMS 2447 x Tx623	W	31.8	49	124	49.69
9078	EMS 2447 x Tx623	W	.	.	.	.
9079	EMS 2447 x Tx623	W	.	.	.	.
9080	EMS 2447 x Tx623	W	.	.	.	.
9065	EMS 2447 x Tx623	B	5.6	47	119	35.86
9066	EMS 2447 x Tx623	B	25.7	47	119	85.48
9067	EMS 2447 x Tx623	B	69.4	47	119	74.15
9068	EMS 2447 x Tx623	B	39.3	43	109	81.45
9069	EMS 2447 x Tx623	B	44.7	50	127	75.98
9070	EMS 2447 x Tx623	B	33.8	51	130	103.43
9071	EMS 2447 x Tx623	B	10.5	51	130	79.31
9072	EMS 2447 x Tx623	B	11.2	49	124	81.76
9073	EMS 2447 x Tx623	B	7.0	53	135	60.97
9074	EMS 2447 x Tx623	B	32.5	53	135	59.87
9075	EMS 2447 x Tx623	B	24.6	44	112	89.52
9076	EMS 2447 x Tx623	B	13.3	46	117	85.75
9077	EMS 2447 x Tx623	B	21.8	50	127	56.92
9078	EMS 2447 x Tx623	B	55.4	48	122	74.86
9079	EMS 2447 x Tx623	B	25.3	47	119	91.47
9080	EMS 2447 x Tx623	B	42.3	56	142	67.73
9165	EMS 2447 x Tx623	B	19.40	52	132	50.65
9166	EMS 2447 x Tx623	B	30.50	52	132	91.52
9167	EMS 2447 x Tx623	B	35.20	50	127	78.14
9168	EMS 2447 x Tx623	B	25.20	58	147	89.01
9169	EMS 2447 x Tx623	B	36.30	55	140	81.38
9170	EMS 2447 x Tx623	B	57.30	47	119	87.37

Plot	Pedigree	FA Test	Chlorophyll	Ht. in.	Height (cm)	Yield (g)
9171	EMS 2447 x Tx623	B	58.60	48	122	50.57
9172	EMS 2447 x Tx623	B	23.80	50	127	89.92
9173	EMS 2447 x Tx623	B	30.90	42	107	90.65
9174	EMS 2447 x Tx623	B	20.10	48	122	84.65
9175	EMS 2447 x Tx623	B	41.20	51	130	92.96
9176	EMS 2447 x Tx623	B	48.90	49	124	59.82
9177	EMS 2447 x Tx623	B	46.50	52	132	91.61
9178	EMS 2447 x Tx623	B	51.10	47	119	80.41
9179	EMS 2447 x Tx623	B	26.40	54	137	90.45
9180	EMS 2447 x Tx623	B	50.30	48	122	88.00
9165	EMS 2447 x Tx623	W	43.60	40	102	16.80
9166	EMS 2447 x Tx623	W	.	.	.	.
9167	EMS 2447 x Tx623	W	49.40	45	114	34.90
9168	EMS 2447 x Tx623	W	.	.	.	.
9169	EMS 2447 x Tx623	W	20.80	46	117	42.44
9170	EMS 2447 x Tx623	W	.	.	.	.
9171	EMS 2447 x Tx623	W	19.40	41	104	.
9172	EMS 2447 x Tx623	W	30.10	41	104	46.52
9173	EMS 2447 x Tx623	W	30.60	48	122	81.91
9174	EMS 2447 x Tx623	W	.	.	.	.
9175	EMS 2447 x Tx623	W	19.10	48	122	50.32
9176	EMS 2447 x Tx623	W	.	.	.	.
9177	EMS 2447 x Tx623	W	30.30	44	112	69.05
9178	EMS 2447 x Tx623	W	16.00	37	94	17.22
9179	EMS 2447 x Tx623	W	49.80	52	132	62.56
9180	EMS 2447 x Tx623	W	.	.	.	.

4.2 Panicle Weight, Aboveground Biomass Weight, Total Biomass (not including

Grain Yield), and Harvest Index (H.I.)

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9001	EMS 2447 mut	W	15.55	63.85	79.40	0.28
9002	Tx2737	B	16.20	119.94	136.14	0.40
9003	B Tx623	B	13.76	146.50	160.26	0.40
9004	915 B	B	14.53	196.74	211.27	0.28
9005	915 B	B	13.14	153.49	166.63	0.31
9006	EMS 2447 mut	W	6.33	25.11	31.44	0.38
9007	B Tx623	B	16.62	145.54	162.16	0.34
9008	Tx2737	B	17.10	82.30	99.40	0.41
9009	B Tx623	B	14.10	133.94	148.04	0.43
9010	915 B	B	12.53	201.67	214.20	0.24
9011	EMS 2447 mut	W	17.99	76.98	94.97	0.27
9012	Tx2737	B	10.40	68.02	78.42	0.42
9013	EMS 2447 mut	W	12.88	67.72	80.60	0.33
9014	B Tx623	B	8.61	104.37	112.98	0.36
9015	Tx2737	B	8.53	57.52	66.05	0.40
9016	915 B	B	15.13	191.61	206.74	0.27
9101	B Tx623	B	7.13	92.00	99.13	0.32
9102	915 B	B	13.36	201.25	214.61	0.26
9103	Tx2737	B	5.00	36.23	41.23	0.47
9104	EMS 2447 mut	W	4.33	30.32	34.65	0.40
9105	915 B	B	15.14	235.84	250.98	0.21
9106	Tx2737	B	10.95	55.55	66.50	0.47
9107	EMS 2447 mut	W	11.01	149.67	160.68	0.09
9108	B Tx623	B	12.43	161.53	161.53	0.37
9109	915 B	B	18.86	282.25	301.11	0.26
9110	EMS 2447 mut	W	11.15	52.05	63.20	0.42
9112	B Tx623	B	8.56	102.12	110.68	0.39
9113	Tx2737	B	10.04	60.54	70.58	0.32
9114	915 B	B	19.13	248.17	267.30	0.24
9115	EMS 2447 mut	W	7.33	47.38	54.71	0.46
9116	B Tx623	B	13.06	147.01	160.07	0.37
9017	Tx2737 x EMS 2447-1	W	.	.	.	.
9018	Tx2737 x EMS 2447-1	W	.	.	.	.
9019	Tx2737 x EMS 2447-1	W	.	.	.	.
9020	Tx2737 x EMS 2447-1	W	.	.	.	.

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9021	Tx2737 x EMS 2447-1	W	.	.	.	.
9022	Tx2737 x EMS 2447-1	W	22.35	147.65	170.00	0.25
9023	Tx2737 x EMS 2447-1	W	12.83	81.43	94.26	0.30
9024	Tx2737 x EMS 2447-1	W	14.15	129.55	143.70	0.24
9025	Tx2737 x EMS 2447-1	W	10.57	79.26	89.83	0.32
9026	Tx2737 x EMS 2447-1	W	17.67	98.45	116.12	0.41
9027	Tx2737 x EMS 2447-1	W	16.62	116.40	133.02	0.38
9028	Tx2737 x EMS 2447-1	W	12.58	75.00	87.58	0.37
9029	Tx2737 x EMS 2447-1	W	12.21	97.35	109.56	0.30
9030	Tx2737 x EMS 2447-1	W	23.75	123.45	147.20	0.32
9031	Tx2737 x EMS 2447-1	W	19.75	79.00	98.75	0.30
9032	Tx2737 x EMS 2447-1	W	10.45	97.57	108.02	0.29
9017	Tx2737 x EMS 2447-1	B	18.25	142.42	160.67	0.44
9018	Tx2737 x EMS 2447-1	B	20.48	111.80	132.28	0.46
9019	Tx2737 x EMS 2447-1	B	21.93	183.25	205.18	0.24
9020	Tx2737 x EMS 2447-1	B	20.08	142.23	162.31	0.44
9021	Tx2737 x EMS 2447-1	B	7.60	66.22	73.82	0.32
9022	Tx2737 x EMS 2447-1	B	12.49	90.63	103.12	0.45
9023	Tx2737 x EMS 2447-1	B	21.58	172.54	194.12	0.38
9024	Tx2737 x EMS 2447-1	B	10.51	86.86	97.37	0.47
9025	Tx2737 x EMS 2447-1	B	13.70	75.66	89.36	0.38
9026	Tx2737 x EMS 2447-1	B	22.06	134.10	156.16	0.44
9027	Tx2737 x EMS 2447-1	B	13.14	92.29	105.43	0.42
9028	Tx2737 x EMS 2447-1	B	10.25	128.24	138.49	0.29
9029	Tx2737 x EMS 2447-1	B	13.29	91.29	104.58	0.39
9030	Tx2737 x EMS 2447-1	B	14.71	117.36	132.07	0.38
9031	Tx2737 x EMS 2447-1	B	17.07	119.27	136.34	0.28
9032	Tx2737 x EMS 2447-1	B	12.69	137.38	150.07	0.29
9117	Tx2737 x EMS 2447-1	B	6.99	90.03	97.02	0.45
9118	Tx2737 x EMS 2447-1	B	18.28	112.91	131.19	0.46
9119	Tx2737 x EMS 2447-1	B	15.40	91.03	106.43	0.50
9120	Tx2737 x EMS 2447-1	B	17.17	128.77	145.94	0.16
9121	Tx2737 x EMS 2447-1	B	12.92	86.30	99.22	0.48
9122	Tx2737 x EMS 2447-1	B	19.24	101.99	121.23	0.47
9123	Tx2737 x EMS 2447-1	B	23.44	113.37	136.81	0.43
9124	Tx2737 x EMS 2447-1	B	10.59	55.12	65.71	0.47
9125	Tx2737 x EMS 2447-1	B	16.68	130.45	147.13	0.39
9126	Tx2737 x EMS 2447-1	B	11.15	77.43	88.58	0.42

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9127	Tx2737 x EMS 2447-1	B	15.57	59.87	75.44	0.51
9128	Tx2737 x EMS 2447-1	B	16.01	90.51	106.52	0.53
9129	Tx2737 x EMS 2447-1	B	12.77	85.44	98.21	0.42
9130	Tx2737 x EMS 2447-1	B	8.15	61.84	69.99	0.45
9131	Tx2737 x EMS 2447-1	B	.	.	.	.
9132	Tx2737 x EMS 2447-1	B	17.60	57.31	74.91	0.53
9117	Tx2737 x EMS 2447-1	W	19.63	91.52	111.15	.
9118	Tx2737 x EMS 2447-1	W	15.63	147.61	163.24	0.22
9119	Tx2737 x EMS 2447-1	W	.	.	.	.
9120	Tx2737 x EMS 2447-1	W	20.65	146.10	166.75	0.03
9121	Tx2737 x EMS 2447-1	W	9.71	61.73	71.44	0.38
9122	Tx2737 x EMS 2447-1	W	.	.	.	.
9123	Tx2737 x EMS 2447-1	W	11.50	61.55	73.05	0.40
9124	Tx2737 x EMS 2447-1	W	16.69	56.37	73.06	0.49
9125	Tx2737 x EMS 2447-1	W	.	.	.	.
9126	Tx2737 x EMS 2447-1	W	7.65	56.65	64.30	0.32
9127	Tx2737 x EMS 2447-1	W	.	.	.	.
9128	Tx2737 x EMS 2447-1	W	.	.	.	.
9129	Tx2737 x EMS 2447-1	W	11.46	61.74	73.20	0.41
9130	Tx2737 x EMS 2447-1	W	27.95	187.01	214.96	.
9131	Tx2737 x EMS 2447-1	W	.	.	.	.
9132	Tx2737 x EMS 2447-1	W	11.47	98.65	110.12	0.41
9033	Tx2737 x EMS 2447-2	W	10.74	64.64	75.38	0.37
9034	Tx2737 x EMS 2447-2	W	11.97	60.87	72.84	0.24
9035	Tx2737 x EMS 2447-2	W	19.29	169.99	189.28	0.27
9036	Tx2737 x EMS 2447-2	W	13.92	90.80	104.72	0.33
9037	Tx2737 x EMS 2447-2	W	12.86	105.61	118.47	0.29
9038	Tx2737 x EMS 2447-2	W	9.94	113.93	123.87	0.24
9039	Tx2737 x EMS 2447-2	W	10.19	78.45	88.64	0.26
9040	Tx2737 x EMS 2447-2	W	11.27	87.64	98.91	0.23
9041	Tx2737 x EMS 2447-2	W	13.13	88.16	101.29	0.36
9042	Tx2737 x EMS 2447-2	W	6.01	80.25	86.26	0.25
9043	Tx2737 x EMS 2447-2	W	8.44	85.46	93.90	0.31
9044	Tx2737 x EMS 2447-2	W	15.32	130.65	145.97	0.28
9045	Tx2737 x EMS 2447-2	W	14.50	143.72	158.22	0.28
9046	Tx2737 x EMS 2447-2	W	11.55	71.46	83.01	0.34
9047	Tx2737 x EMS 2447-2	W	11.94	73.16	85.10	0.36
9048	Tx2737 x EMS 2447-2	W	.	.	.	.

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9033	Tx2737 x EMS 2447-2	B	13.73	73.07	86.80	0.44
9034	Tx2737 x EMS 2447-2	B	13.11	100.75	113.86	0.42
9035	Tx2737 x EMS 2447-2	B	14.47	172.00	186.47	0.22
9036	Tx2737 x EMS 2447-2	B	16.60	142.91	159.51	0.30
9037	Tx2737 x EMS 2447-2	B	11.61	100.88	112.49	0.43
9038	Tx2737 x EMS 2447-2	B	9.47	116.37	125.84	0.36
9039	Tx2737 x EMS 2447-2	B	13.34	127.00	140.34	0.27
9040	Tx2737 x EMS 2447-2	B	13.70	110.78	124.48	0.43
9041	Tx2737 x EMS 2447-2	B	10.49	72.79	83.28	0.38
9042	Tx2737 x EMS 2447-2	B	19.81	127.12	146.93	0.47
9043	Tx2737 x EMS 2447-2	B	13.90	103.81	117.71	0.43
9044	Tx2737 x EMS 2447-2	B	10.10	112.60	122.70	0.30
9045	Tx2737 x EMS 2447-2	B	26.91	111.51	138.42	0.28
9046	Tx2737 x EMS 2447-2	B	12.70	119.73	132.43	0.37
9047	Tx2737 x EMS 2447-2	B	13.80	220.53	234.33	0.15
9048	Tx2737 x EMS 2447-2	B	18.73	103.10	121.83	0.45
9133	Tx2737 x EMS 2447-2	B	17.81	111.97	129.78	0.44
9134	Tx2737 x EMS 2447-2	B	12.97	128.03	141.00	0.35
9135	Tx2737 x EMS 2447-2	B	19.98	169.75	189.73	0.40
9136	Tx2737 x EMS 2447-2	B	14.38	127.46	141.84	0.38
9137	Tx2737 x EMS 2447-2	B	14.56	92.51	107.07	0.37
9138	Tx2737 x EMS 2447-2	B	15.90	77.60	93.50	0.47
9139	Tx2737 x EMS 2447-2	B	11.82	72.24	84.06	0.39
9140	Tx2737 x EMS 2447-2	B	15.74	110.86	126.60	0.36
9141	Tx2737 x EMS 2447-2	B	9.08	107.19	116.27	0.37
9142	Tx2737 x EMS 2447-2	B	19.45	120.58	140.03	0.42
9143	Tx2737 x EMS 2447-2	B	18.08	60.80	78.88	0.48
9144	Tx2737 x EMS 2447-2	B	14.40	86.82	101.22	0.32
9145	Tx2737 x EMS 2447-2	B	14.18	79.79	93.97	0.44
9146	Tx2737 x EMS 2447-2	B	11.87	100.95	112.82	0.36
9147	Tx2737 x EMS 2447-2	B	7.45	69.56	77.01	0.42
9148	Tx2737 x EMS 2447-2	B	15.63	115.68	131.31	0.37
9133	Tx2737 x EMS 2447-2	B	11.12	52.20	63.32	0.43
9134	Tx2737 x EMS 2447-2	W	7.75	89.55	97.30	0.41
9135	Tx2737 x EMS 2447-2	W	15.65	164.35	180.00	.
9136	Tx2737 x EMS 2447-2	W	12.67	88.00	100.67	0.39
9137	Tx2737 x EMS 2447-2	W	12.98	112.72	125.70	0.37
9138	Tx2737 x EMS 2447-2	W	.	.	.	.

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9139	Tx2737 x EMS 2447-2	W	4.79	63.17	67.96	0.40
9140	Tx2737 x EMS 2447-2	W	7.65	85.00	92.65	0.35
9141	Tx2737 x EMS 2447-2	W	5.02	65.58	70.60	0.37
9142	Tx2737 x EMS 2447-2	W	15.30	82.08	97.38	0.35
9143	Tx2737 x EMS 2447-2	W	18.68	95.92	114.60	0.37
9144	Tx2737 x EMS 2447-2	W	20.35	81.05	101.40	0.36
9145	Tx2737 x EMS 2447-2	W	9.47	35.76	45.23	0.35
9146	Tx2737 x EMS 2447-2	W	8.42	106.22	114.64	0.33
9147	Tx2737 x EMS 2447-2	W	12.64	136.10	148.74	0.20
9148	Tx2737 x EMS 2447-2	W	.	.	.	.
9149	EMS 2447 x 915B	B	18.77	200.29	219.06	0.31
9150	EMS 2447 x 915B	B	8.52	203.89	212.41	0.17
9151	EMS 2447 x 915B	B	22.33	259.95	282.28	0.28
9152	EMS 2447 x 915B	B	10.89	209.35	220.24	0.10
9153	EMS 2447 x 915B	B	23.94	293.59	317.53	0.26
9154	EMS 2447 x 915B	B	16.43	306.78	323.21	0.03
9155	EMS 2447 x 915B	B	8.50	158.20	166.70	0.27
9156	EMS 2447 x 915B	B	16.15	212.78	228.93	0.28
9157	EMS 2447 x 915B	B	10.85	92.75	103.60	0.31
9158	EMS 2447 x 915B	B	16.64	216.17	232.81	0.25
9159	EMS 2447 x 915B	B	16.88	306.77	323.65	0.23
9160	EMS 2447 x 915B	B	12.18	128.64	140.82	0.36
9161	EMS 2447 x 915B	B	16.62	176.23	192.85	0.35
9162	EMS 2447 x 915B	B	7.48	150.65	158.13	0.23
9163	EMS 2447 x 915B	B	16.07	207.56	223.63	0.31
9164	EMS 2447 x 915B	B	18.75	133.54	152.29	0.40
9149	EMS 2447 x 915B	W	16.02	192.89	208.91	0.30
9150	EMS 2447 x 915B	W	31.20	253.36	284.56	0.35
9151	EMS 2447 x 915B	W	24.38	524.68	549.06	.
9152	EMS 2447 x 915B	W	19.74	351.86	371.60	.
9153	EMS 2447 x 915B	W	10.78	149.89	160.67	0.27
9154	EMS 2447 x 915B	W	13.67	199.76	213.43	0.30
9155	EMS 2447 x 915B	W	15.67	187.37	203.04	0.37
9156	EMS 2447 x 915B	W	6.55	63.86	70.41	0.32
9157	EMS 2447 x 915B	W	.	.	.	.
9158	EMS 2447 x 915B	W	13.66	198.77	212.43	0.23
9159	EMS 2447 x 915B	W	5.37	145.92	151.29	0.33
9160	EMS 2447 x 915B	W	12.43	222.69	235.12	.

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9161	EMS 2447 x 915B	W	.	.	.	.
9162	EMS 2447 x 915B	W	.	104.53	104.53	.
9163	EMS 2447 x 915B	W	13.00	140.86	153.86	0.31
9164	EMS 2447 x 915B	W	19.50	258.81	278.31	0.15
9049	EMS 2447 x 915B	W	14.80	170.61	185.41	0.25
9050	EMS 2447 x 915B	W	8.05	58.15	66.20	0.43
9051	EMS 2447 x 915B	W	.	.	.	.
9052	EMS 2447 x 915B	W	17.09	313.33	330.42	0.18
9053	EMS 2447 x 915B	W	6.91	98.86	105.77	0.21
9054	EMS 2447 x 915B	W	12.32	271.83	284.15	0.16
9055	EMS 2447 x 915B	W	7.72	141.47	149.19	0.06
9056	EMS 2447 x 915B	W	7.25	92.99	100.24	0.20
9057	EMS 2447 x 915B	W	7.15	132.30	139.45	0.27
9058	EMS 2447 x 915B	W	8.58	129.78	138.36	0.29
9059	EMS 2447 x 915B	W	15.41	252.48	267.89	.
9060	EMS 2447 x 915B	W	10.77	143.68	154.45	0.20
9061	EMS 2447 x 915B	W	15.82	191.50	207.32	0.26
9062	EMS 2447 x 915B	W	20.52	363.85	384.37	0.14
9063	EMS 2447 x 915B	W	23.31	248.37	271.68	0.24
9064	EMS 2447 x 915B	W	10.22	122.00	132.22	0.25
9049	EMS 2447 x 915B	B	16.13	212.46	228.59	0.29
9050	EMS 2447 x 915B	B	15.12	184.56	199.68	0.31
9051	EMS 2447 x 915B	B	15.98	258.75	274.73	0.18
9052	EMS 2447 x 915B	B	27.73	345.62	373.35	0.26
9053	EMS 2447 x 915B	B	9.16	154.00	163.16	0.26
9054	EMS 2447 x 915B	B	13.85	252.22	266.07	0.16
9055	EMS 2447 x 915B	B	12.37	180.28	192.65	0.28
9056	EMS 2447 x 915B	B	19.19	320.95	340.14	0.18
9057	EMS 2447 x 915B	B	23.95	275.96	299.91	0.24
9058	EMS 2447 x 915B	B	14.50	329.71	344.21	0.26
9059	EMS 2447 x 915B	B	21.79	306.81	328.60	0.21
9060	EMS 2447 x 915B	B	11.37	169.86	181.23	0.23
9061	EMS 2447 x 915B	B	12.32	314.77	327.09	0.24
9062	EMS 2447 x 915B	B	13.75	203.84	217.59	0.36
9063	EMS 2447 x 915B	B	13.97	189.00	202.97	0.35
9064	EMS 2447 x 915B	B	14.98	203.04	218.02	0.17
9065	EMS 2447 x Tx623	W	.	.	.	.
9066	EMS 2447 x Tx623	W	16.93	85.43	102.36	0.33

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9067	EMS 2447 x Tx623	W	.	.	.	.
9068	EMS 2447 x Tx623	W	8.23	57.88	66.11	0.17
9069	EMS 2447 x Tx623	W	12.33	101.96	114.29	0.31
9070	EMS 2447 x Tx623	W	.	.	.	.
9071	EMS 2447 x Tx623	W	.	.	.	.
9072	EMS 2447 x Tx623	W	13.92	106.40	120.32	0.14
9073	EMS 2447 x Tx623	W	9.20	82.45	91.65	0.40
9074	EMS 2447 x Tx623	W	27.99	150.33	178.32	0.35
9075	EMS 2447 x Tx623	W	12.75	215.96	228.71	0.19
9076	EMS 2447 x Tx623	W	11.46	95.07	106.53	0.22
9077	EMS 2447 x Tx623	W	17.28	124.37	141.65	0.26
9078	EMS 2447 x Tx623	W	.	.	.	.
9079	EMS 2447 x Tx623	W	.	.	.	.
9080	EMS 2447 x Tx623	W	.	.	.	.
9065	EMS 2447 x Tx623	B	17.70	65.39	83.09	0.30
9066	EMS 2447 x Tx623	B	18.36	141.53	159.89	0.35
9067	EMS 2447 x Tx623	B	13.99	116.86	130.85	0.36
9068	EMS 2447 x Tx623	B	17.47	118.93	136.40	0.37
9069	EMS 2447 x Tx623	B	16.57	147.34	163.91	0.32
9070	EMS 2447 x Tx623	B	35.03	188.05	223.08	0.32
9071	EMS 2447 x Tx623	B	14.93	143.77	158.70	0.33
9072	EMS 2447 x Tx623	B	15.79	125.57	141.36	0.37
9073	EMS 2447 x Tx623	B	12.83	110.36	123.19	0.33
9074	EMS 2447 x Tx623	B	11.95	85.92	97.87	0.38
9075	EMS 2447 x Tx623	B	17.15	125.80	142.95	0.39
9076	EMS 2447 x Tx623	B	14.90	113.68	128.58	0.40
9077	EMS 2447 x Tx623	B	23.04	84.87	107.91	0.35
9078	EMS 2447 x Tx623	B	25.05	143.53	168.58	0.31
9079	EMS 2447 x Tx623	B	14.20	111.74	125.94	0.42
9080	EMS 2447 x Tx623	B	14.30	132.21	146.51	0.32
9165	EMS 2447 x Tx623	B	10.70	42.91	53.61	0.49
9166	EMS 2447 x Tx623	B	10.92	.	.	.
9167	EMS 2447 x Tx623	B	13.35	65.88	79.23	0.50
9168	EMS 2447 x Tx623	B	14.97	102.28	117.25	0.43
9169	EMS 2447 x Tx623	B	13.01	143.51	156.52	0.34
9170	EMS 2447 x Tx623	B	18.14	109.45	127.59	0.41
9171	EMS 2447 x Tx623	B	9.92	83.01	92.93	0.35
9172	EMS 2447 x Tx623	B	19.44	128.56	148.00	0.38

Plot	Pedigree	FA Test	Panicle wt. (g)	Biomass (g)	Total Biomass (g)	H.I.
9173	EMS 2447 x Tx623	B	19.72	118.05	137.77	0.40
9174	EMS 2447 x Tx623	B	11.74	107.38	119.12	0.42
9175	EMS 2447 x Tx623	B	17.02	96.21	113.23	0.45
9176	EMS 2447 x Tx623	B	14.46	101.32	115.78	0.34
9177	EMS 2447 x Tx623	B	24.41	125.86	150.27	0.38
9178	EMS 2447 x Tx623	B	15.06	99.38	114.44	0.41
9179	EMS 2447 x Tx623	B	11.58	.	.	.
9180	EMS 2447 x Tx623	B	22.68	99.87	122.55	0.42
9165	EMS 2447 x Tx623	W	4.31	80.43	84.74	0.17
9166	EMS 2447 x Tx623	W	.	.	.	.
9167	EMS 2447 x Tx623	W	14.32	101.96	116.28	0.23
9168	EMS 2447 x Tx623	W	.	.	.	.
9169	EMS 2447 x Tx623	W	16.37	65.13	81.50	0.34
9170	EMS 2447 x Tx623	W	.	.	.	.
9171	EMS 2447 x Tx623	W	9.10	75.91	85.01	.
9172	EMS 2447 x Tx623	W	13.04	91.03	104.07	0.31
9173	EMS 2447 x Tx623	W	14.46	163.41	177.87	0.32
9174	EMS 2447 x Tx623	W	.	.	.	.
9175	EMS 2447 x Tx623	W	7.95	75.44	83.39	0.38
9176	EMS 2447 x Tx623	W	.	.	.	.
9177	EMS 2447 x Tx623	W	14.56	128.55	143.11	0.33
9178	EMS 2447 x Tx623	W	9.54	34.55	44.09	0.28
9179	EMS 2447 x Tx623	W	11.06	90.63	101.69	0.38
9180	EMS 2447 x Tx623	W	.	.	.	.

5. <u>Stay-Green Experiment Sample 1 Data</u>

Rep	Pedigree	Chlorophyll Meter Reading
1	BTx623	19.2
2	BTx623	25.9
3	BTx623	20.2
4	BTx623	32.1
5	BTx623	30.1
6	BTx623	24.6
7	BTx623	24.6
8	BTx623	17.0
9	BTx623	15.2
10	BTx623	14.4
11	BTx623	18.9
12	BTx623	25.5
13	BTx623	15.8
14	BTx623	21.5
15	BTx623	27.1
16	BTx623	30.2
17	BTx623	13.5
18	BTx623	19.4
19	BTx623	17.2
20	BTx623	18.9
21	BTx623	15.9
22	BTx623	18.3
23	BTx623	15.4
24	BTx623	26.0
25	BTx623	28.5
26	BTx623	22.9
27	BTx623	24.1
28	BTx623	30.4
29	BTx623	30.8
1	EMS 932	19.5
2	EMS 932	25.9
3	EMS 932	30.8
4	EMS 932	21.4
5	EMS 932	28.4
6	EMS 932	25.5
7	EMS 932	21.7

Rep	Pedigree	Chlorophyll Meter Reading
8	EMS 932	15.6
9	EMS 932	24.7
10	EMS 932	19.8
11	EMS 932	18.0
12	EMS 932	21.3
13	EMS 932	22.8
14	EMS 932	20.5
15	EMS 932	19.0
16	EMS 932	25.7
17	EMS 932	16.3
18	EMS 932	19.3
19	EMS 932	17.7
20	EMS 932	19.9
21	EMS 932	18.7
22	EMS 932	17.9
23	EMS 932	24.2
24	EMS 932	27.4
25	EMS 932	28.3
26	EMS 932	23.2
27	EMS 932	18.3
28	EMS 932	30.5
29	EMS 932	22.9
1	EMS 5085 mut x Tx623 F3	19.0
2	EMS 5085 mut x Tx623 F3	9.9
3	EMS 5085 mut x Tx623 F3	22.7
4	EMS 5085 mut x Tx623 F3	23.1
5	EMS 5085 mut x Tx623 F3	27.2
6	EMS 5085 mut x Tx623 F3	18.5
7	EMS 5085 mut x Tx623 F3	23.9
8	EMS 5085 mut x Tx623 F3	17.1
9	EMS 5085 mut x Tx623 F3	23.8
10	EMS 5085 mut x Tx623 F3	15.7
11	EMS 5085 mut x Tx623 F3	18.2
12	EMS 5085 mut x Tx623 F3	22.0
13	EMS 5085 mut x Tx623 F3	20.0
14	EMS 5085 mut x Tx623 F3	25.7
15	EMS 5085 mut x Tx623 F3	28.4

Rep	Pedigree	Chlorophyll Meter Reading
16	EMS 5085 mut x Tx623 F3	29.1
17	EMS 5085 mut x Tx623 F3	11.9
18	EMS 5085 mut x Tx623 F3	24.2
19	EMS 5085 mut x Tx623 F3	21.4
20	EMS 5085 mut x Tx623 F3	20.8
21	EMS 5085 mut x Tx623 F3	21.8
22	EMS 5085 mut x Tx623 F3	25.8
23	EMS 5085 mut x Tx623 F3	19.0
24	EMS 5085 mut x Tx623 F3	24.5
25	EMS 5085 mut x Tx623 F3	23.8
26	EMS 5085 mut x Tx623 F3	24.1
27	EMS 5085 mut x Tx623 F3	29.7
28	EMS 5085 mut x Tx623 F3	34.1
29	EMS 5085 mut x Tx623 F3	19.5

6. <u>Stay-Green Experiment Sample 2 Data</u>

Rep	Pedigree	Chlorophyll Meter Reading
1	BTx623	15.4
2	BTx623	47.1
3	BTx623	33.2
4	BTx623	34.9
5	BTx623	33.1
6	BTx623	30.4
7	BTx623	28.6
8	BTx623	20.4
9	BTx623	24.7
10	BTx623	18.9
11	BTx623	24.2
12	BTx623	22.3
13	BTx623	17.9
14	BTx623	23.4
15	BTx623	22.4
16	BTx623	25.6
17	BTx623	16.6
18	BTx623	22.2
19	BTx623	18.7
20	BTx623	18.4
21	BTx623	21.4
22	BTx623	13.7
23	BTx623	16.4
24	BTx623	32.7
25	BTx623	29.4
26	BTx623	30.5
27	BTx623	31.8
28	BTx623	23.2
29	BTx623	36.6
1	EMS 932	25.1
2	EMS 932	29.6
3	EMS 932	31.2
4	EMS 932	29.2
5	EMS 932	34.9

Rep	Pedigree	Chlorophyll Meter Reading
6	EMS 932	29.8
7	EMS 932	31.7
8	EMS 932	34.2
9	EMS 932	34.3
10	EMS 932	14.6
11	EMS 932	18.8
12	EMS 932	28.4
13	EMS 932	26.7
14	EMS 932	22.2
15	EMS 932	23.5
16	EMS 932	19.6
17	EMS 932	25.8
18	EMS 932	22.4
19	EMS 932	19.2
20	EMS 932	21.5
21	EMS 932	21.1
22	EMS 932	22.1
23	EMS 932	21.8
24	EMS 932	30.3
25	EMS 932	23.7
26	EMS 932	37.4
27	EMS 932	26.3
28	EMS 932	24.1
29	EMS 932	23.8
1	EMS 5085 mut x Tx623 F3	23.1
2	EMS 5085 mut x Tx623 F3	16.2
3	EMS 5085 mut x Tx623 F3	25.9
4	EMS 5085 mut x Tx623 F3	37.3
5	EMS 5085 mut x Tx623 F3	32.1
6	EMS 5085 mut x Tx623 F3	37.4
7	EMS 5085 mut x Tx623 F3	27.6
8	EMS 5085 mut x Tx623 F3	24.7
9	EMS 5085 mut x Tx623 F3	37.3
10	EMS 5085 mut x Tx623 F3	16.4
11	EMS 5085 mut x Tx623 F3	15.9
12	EMS 5085 mut x Tx623 F3	33.0
13	EMS 5085 mut x Tx623 F3	19.2

Rep	Pedigree	Chlorophyll Meter Reading
14	EMS 5085 mut x Tx623 F3	29.2
15	EMS 5085 mut x Tx623 F3	27.0
16	EMS 5085 mut x Tx623 F3	39.4
17	EMS 5085 mut x Tx623 F3	12.8
18	EMS 5085 mut x Tx623 F3	21.5
19	EMS 5085 mut x Tx623 F3	18.6
20	EMS 5085 mut x Tx623 F3	31.8
21	EMS 5085 mut x Tx623 F3	17.7
22	EMS 5085 mut x Tx623 F3	23.8
23	EMS 5085 mut x Tx623 F3	15.3
24	EMS 5085 mut x Tx623 F3	28.0
25	EMS 5085 mut x Tx623 F3	32.9
26	EMS 5085 mut x Tx623 F3	32.5
27	EMS 5085 mut x Tx623 F3	47.4
28	EMS 5085 mut x Tx623 F3	26.7
29	EMS 5085 mut x Tx623 F3	24.1

7. <u>Stay-Green Experiment Sample 3 Data</u>

Rep	Pedigree	Chlorophyll Meter Reading
1	BTx623	27.9
2	BTx623	34.3
3	BTx623	38.8
4	BTx623	28.8
5	BTx623	35.8
6	BTx623	32.6
7	BTx623	27.3
8	BTx623	24.3
9	BTx623	26.8
10	BTx623	16.0
11	BTx623	19.4
12	BTx623	26.1
13	BTx623	17.9
14	BTx623	24.5
15	BTx623	33.0
16	BTx623	24.1
17	BTx623	16.2
18	BTx623	19.6
19	BTx623	15.6
20	BTx623	18.7
21	BTx623	15.3
22	BTx623	10.3
23	BTx623	10.9
24	BTx623	26.6
25	BTx623	18.5
26	BTx623	21.4
27	BTx623	26.0
28	BTx623	13.0
29	BTx623	17.8
1	EMS 932	16.7
2	EMS 932	30.8
3	EMS 932	38.3
4	EMS 932	26.7
5	EMS 932	35.3

Rep	Pedigree	Chlorophyll Meter Reading
6	EMS 932	18.7
7	EMS 932	20.5
8	EMS 932	27.3
9	EMS 932	29.0
10	EMS 932	14.4
11	EMS 932	12.5
12	EMS 932	25.3
13	EMS 932	27.5
14	EMS 932	35.1
15	EMS 932	21.9
16	EMS 932	31.8
17	EMS 932	22.5
18	EMS 932	17.2
19	EMS 932	14.6
20	EMS 932	13.2
21	EMS 932	10.7
22	EMS 932	16.8
23	EMS 932	11.5
24	EMS 932	31.3
25	EMS 932	20.7
26	EMS 932	18.7
27	EMS 932	28.5
28	EMS 932	24.0
29	EMS 932	12.4
1	EMS 5085 mut x Tx623 F3	28.6
2	EMS 5085 mut x Tx623 F3	25.7
3	EMS 5085 mut x Tx623 F3	38.6
4	EMS 5085 mut x Tx623 F3	28.9
5	EMS 5085 mut x Tx623 F3	31.4
6	EMS 5085 mut x Tx623 F3	32.3
7	EMS 5085 mut x Tx623 F3	24.9
8	EMS 5085 mut x Tx623 F3	24.6
9	EMS 5085 mut x Tx623 F3	22.7
10	EMS 5085 mut x Tx623 F3	10.9
11	EMS 5085 mut x Tx623 F3	9.1
12	EMS 5085 mut x Tx623 F3	32.4
13	EMS 5085 mut x Tx623 F3	23.8

Rep	Pedigree	Chlorophyll Meter Reading
14	EMS 5085 mut x Tx623 F3	27.5
15	EMS 5085 mut x Tx623 F3	28.2
16	EMS 5085 mut x Tx623 F3	27.9
17	EMS 5085 mut x Tx623 F3	13.5
18	EMS 5085 mut x Tx623 F3	20.1
19	EMS 5085 mut x Tx623 F3	10.8
20	EMS 5085 mut x Tx623 F3	16.7
21	EMS 5085 mut x Tx623 F3	13.5
22	EMS 5085 mut x Tx623 F3	17.3
23	EMS 5085 mut x Tx623 F3	14.5
24	EMS 5085 mut x Tx623 F3	25.5
25	EMS 5085 mut x Tx623 F3	33.1
26	EMS 5085 mut x Tx623 F3	19.4
27	EMS 5085 mut x Tx623 F3	25.7
28	EMS 5085 mut x Tx623 F3	12.7
29	EMS 5085 mut x Tx623 F3	19.3

8. Stay-Green Experiment Sample 4 Data

Rep	Pedigree	Chlorophyll Meter Reading
1	BTx623	43.0
2	BTx623	38.5
3	BTx623	34.2
4	BTx623	20.2
5	BTx623	24.6
6	BTx623	30.7
7	BTx623	17.9
8	BTx623	18.6
9	BTx623	26.0
10	BTx623	22.1
11	BTx623	23.7
12	BTx623	22.3
13	BTx623	28.9
14	BTx623	23.6
15	BTx623	27.6
16	BTx623	33.8
17	BTx623	28.6
18	BTx623	24.2
19	BTx623	28.8
20	BTx623	19.4
21	BTx623	30.1
22	BTx623	23.2
23	BTx623	21.2
24	BTx623	22.6
25	BTx623	21.6
26	BTx623	24.6
27	BTx623	21.8
28	BTx623	19.6
29	BTx623	18.2
1	EMS 932	26.2
2	EMS 932	22.3
3	EMS 932	30.5
4	EMS 932	21.3
5	EMS 932	17.8
6	EMS 932	26.3
7	EMS 932	22.9

Rep	Pedigree	Chlorophyll Meter Reading
8	EMS 932	17.4
9	EMS 932	27.6
10	EMS 932	25.3
11	EMS 932	24.0
12	EMS 932	29.8
13	EMS 932	22.6
14	EMS 932	31.3
15	EMS 932	29.9
16	EMS 932	33.7
17	EMS 932	24.4
18	EMS 932	21.1
19	EMS 932	23.8
20	EMS 932	22.7
21	EMS 932	14.5
22	EMS 932	13.9
23	EMS 932	18.5
24	EMS 932	26.1
25	EMS 932	22.6
26	EMS 932	17.3
27	EMS 932	19.0
28	EMS 932	20.7
29	EMS 932	16.0
1	EMS 5085 mut x Tx623 F3	29.9
2	EMS 5085 mut x Tx623 F3	26.8
3	EMS 5085 mut x Tx623 F3	36.6
4	EMS 5085 mut x Tx623 F3	25.8
5	EMS 5085 mut x Tx623 F3	18.3
6	EMS 5085 mut x Tx623 F3	23.5
7	EMS 5085 mut x Tx623 F3	18.9
8	EMS 5085 mut x Tx623 F3	18.2
9	EMS 5085 mut x Tx623 F3	29.2
10	EMS 5085 mut x Tx623 F3	16.6
11	EMS 5085 mut x Tx623 F3	22.8
12	EMS 5085 mut x Tx623 F3	29.0
13	EMS 5085 mut x Tx623 F3	18.2
14	EMS 5085 mut x Tx623 F3	21.8
15	EMS 5085 mut x Tx623 F3	21.6

Rep	Pedigree	Chlorophyll Meter Reading
16	EMS 5085 mut x Tx623 F3	29.1
17	EMS 5085 mut x Tx623 F3	30.3
18	EMS 5085 mut x Tx623 F3	28.9
19	EMS 5085 mut x Tx623 F3	31.3
20	EMS 5085 mut x Tx623 F3	27.7
21	EMS 5085 mut x Tx623 F3	17.7
22	EMS 5085 mut x Tx623 F3	14.4
23	EMS 5085 mut x Tx623 F3	29.9
24	EMS 5085 mut x Tx623 F3	14.7
25	EMS 5085 mut x Tx623 F3	18.7
26	EMS 5085 mut x Tx623 F3	20.6
27	EMS 5085 mut x Tx623 F3	16.0
28	EMS 5085 mut x Tx623 F3	25.6
29	EMS 5085 mut x Tx623 F3	17.2

9. <u>Stay-Green Experiment Sample 5 Data</u>

Rep	Pedigree	Chlorophyll Meter Reading
1	BTx623	38.7
2	BTx623	42.5
3	BTx623	42.2
4	BTx623	37.0
5	BTx623	38.4
6	BTx623	34.8
7	BTx623	35.4
8	BTx623	37.5
9	BTx623	40.5
10	BTx623	40.1
11	BTx623	45.3
12	BTx623	30.3
13	BTx623	30.8
14	BTx623	29.7
15	BTx623	35.0
16	BTx623	33.4
17	BTx623	34.9
18	BTx623	30.2
19	BTx623	33.8
20	BTx623	42.7
21	BTx623	36.6
22	BTx623	35.7
23	BTx623	30.9
24	BTx623	37.1
25	BTx623	30.4
26	BTx623	50.9
27	BTx623	36.5
28	BTx623	41.1
29	BTx623	40.4
1	EMS 932	28.3
2	EMS 932	32.3
3	EMS 932	32.4
4	EMS 932	28.0
5	EMS 932	34.6
6	EMS 932	34.0
7	EMS 932	39.2

Rep	Pedigree	Chlorophyll Meter Reading
8	EMS 932	34.9
9	EMS 932	33.1
10	EMS 932	29.0
11	EMS 932	25.5
12	EMS 932	34.3
13	EMS 932	29.8
14	EMS 932	36.1
15	EMS 932	34.4
16	EMS 932	36.8
17	EMS 932	28.4
18	EMS 932	39.5
19	EMS 932	28.9
20	EMS 932	29.6
21	EMS 932	31.6
22	EMS 932	34.2
23	EMS 932	38.0
24	EMS 932	31.1
25	EMS 932	38.4
26	EMS 932	26.1
27	EMS 932	38.2
28	EMS 932	47.6
29	EMS 932	32.6
1	EMS 5085 mut x Tx623 F3	39.5
2	EMS 5085 mut x Tx623 F3	23.7
3	EMS 5085 mut x Tx623 F3	34.0
4	EMS 5085 mut x Tx623 F3	35.9
5	EMS 5085 mut x Tx623 F3	41.9
6	EMS 5085 mut x Tx623 F3	32.3
7	EMS 5085 mut x Tx623 F3	33.3
8	EMS 5085 mut x Tx623 F3	40.2
9	EMS 5085 mut x Tx623 F3	36.3
10	EMS 5085 mut x Tx623 F3	35.2
11	EMS 5085 mut x Tx623 F3	38.4
12	EMS 5085 mut x Tx623 F3	33.7
13	EMS 5085 mut x Tx623 F3	29.2
14	EMS 5085 mut x Tx623 F3	17.6
15	EMS 5085 mut x Tx623 F3	30.5

Rep	Pedigree	Chlorophyll Meter Reading
16	EMS 5085 mut x Tx623 F3	46.3
17	EMS 5085 mut x Tx623 F3	27.1
18	EMS 5085 mut x Tx623 F3	31.0
19	EMS 5085 mut x Tx623 F3	30.4
20	EMS 5085 mut x Tx623 F3	34.4
21	EMS 5085 mut x Tx623 F3	24.6
22	EMS 5085 mut x Tx623 F3	40.4
23	EMS 5085 mut x Tx623 F3	29.7
24	EMS 5085 mut x Tx623 F3	31.9
25	EMS 5085 mut x Tx623 F3	47.8
26	EMS 5085 mut x Tx623 F3	27.7
27	EMS 5085 mut x Tx623 F3	32.1
28	EMS 5085 mut x Tx623 F3	37.8
29	EMS 5085 mut x Tx623 F3	28.1

10. <u>Stay-Green Experiment Sample 6 Data</u>

Rep	Pedigree	Chlorophyll Meter Reading
1	BTx623	37.1
2	BTx623	34.2
3	BTx623	41.1
4	BTx623	32.9
5	BTx623	33.0
6	BTx623	39.6
7	BTx623	28.3
8	BTx623	59.4
9	BTx623	41.0
10	BTx623	48.0
11	BTx623	46.8
12	BTx623	40.8
13	BTx623	44.7
14	BTx623	37.1
15	BTx623	48.7
16	BTx623	43.2
17	BTx623	50.7
18	BTx623	51.6
19	BTx623	39.9
20	BTx623	45.0
21	BTx623	45.9
22	BTx623	38.6
23	BTx623	38.6
24	BTx623	41.1
25	BTx623	44.5
26	BTx623	41.1
27	BTx623	34.9
28	BTx623	39.8
29	BTx623	23.7
1	EMS 932	38.5
2	EMS 932	34.1
3	EMS 932	31.3
4	EMS 932	27.2
5	EMS 932	34.8
6	EMS 932	28.8
7	EMS 932	47.7

Rep	Pedigree	Chlorophyll Meter Reading
8	EMS 932	30.5
9	EMS 932	38.9
10	EMS 932	26.8
11	EMS 932	41.7
12	EMS 932	38.4
13	EMS 932	29.9
14	EMS 932	28.1
15	EMS 932	39.0
16	EMS 932	40.8
17	EMS 932	36.4
18	EMS 932	42.9
19	EMS 932	38.4
20	EMS 932	29.9
21	EMS 932	37.0
22	EMS 932	31.2
23	EMS 932	40.1
24	EMS 932	29.9
25	EMS 932	31.7
26	EMS 932	33.6
27	EMS 932	27.6
28	EMS 932	28.9
29	EMS 932	29.9
1	EMS 5085 mut x Tx623 F3	29.7
2	EMS 5085 mut x Tx623 F3	31.3
3	EMS 5085 mut x Tx623 F3	33.5
4	EMS 5085 mut x Tx623 F3	28.9
5	EMS 5085 mut x Tx623 F3	35.2
6	EMS 5085 mut x Tx623 F3	32.4
7	EMS 5085 mut x Tx623 F3	41.0
8	EMS 5085 mut x Tx623 F3	27.4
9	EMS 5085 mut x Tx623 F3	41.7
10	EMS 5085 mut x Tx623 F3	30.5
11	EMS 5085 mut x Tx623 F3	37.8
12	EMS 5085 mut x Tx623 F3	36.9
13	EMS 5085 mut x Tx623 F3	31.5
14	EMS 5085 mut x Tx623 F3	26.9
15	EMS 5085 mut x Tx623 F3	39.8

Rep	Pedigree	Chlorophyll Meter Reading
16	EMS 5085 mut x Tx623 F3	41.4
17	EMS 5085 mut x Tx623 F3	33.5
18	EMS 5085 mut x Tx623 F3	31.5
19	EMS 5085 mut x Tx623 F3	50.4
20	EMS 5085 mut x Tx623 F3	42.7
21	EMS 5085 mut x Tx623 F3	32.2
22	EMS 5085 mut x Tx623 F3	44.8
23	EMS 5085 mut x Tx623 F3	40.5
24	EMS 5085 mut x Tx623 F3	38.3
25	EMS 5085 mut x Tx623 F3	39.8
26	EMS 5085 mut x Tx623 F3	43.6
27	EMS 5085 mut x Tx623 F3	24.2
28	EMS 5085 mut x Tx623 F3	44.2
29	EMS 5085 mut x Tx623 F3	43.7

11. <u>Stay-Green Experiment Sample 7 Data</u>

Rep	Pedigree	Chlorophyll Meter Reading
1	BTx623	32.0
2	BTx623	24.8
3	BTx623	22.7
4	BTx623	21.0
5	BTx623	25.9
6	BTx623	26.8
7	BTx623	30.2
8	BTx623	47.6
9	BTx623	27.7
10	BTx623	28.6
11	BTx623	30.0
12	BTx623	25.5
13	BTx623	24.5
14	BTx623	24.3
15	BTx623	48.8
16	BTx623	32.7
17	BTx623	38.0
18	BTx623	28.5
19	BTx623	28.3
20	BTx623	34.4
21	BTx623	34.3
22	BTx623	30.9
23	BTx623	27.6
24	BTx623	44.6
25	BTx623	16.6
26	BTx623	34.6
27	BTx623	17.8
28	BTx623	15.6
29	BTx623	16.1
1	EMS 932	30.0
2	EMS 932	27.3
3	EMS 932	19.0
4	EMS 932	12.7
5	EMS 932	15.2
6	EMS 932	27.4
7	EMS 932	30.0

Rep	Pedigree	Chlorophyll Meter Reading
8	EMS 932	29.3
9	EMS 932	28.2
10	EMS 932	40.2
11	EMS 932	25.7
12	EMS 932	16.0
13	EMS 932	23.9
14	EMS 932	32.3
15	EMS 932	19.7
16	EMS 932	24.8
17	EMS 932	32.3
18	EMS 932	35.3
19	EMS 932	30.2
20	EMS 932	16.5
21	EMS 932	20.5
22	EMS 932	29.9
23	EMS 932	18.9
24	EMS 932	20.3
25	EMS 932	30.1
26	EMS 932	29.3
27	EMS 932	16.5
28	EMS 932	19.6
29	EMS 932	30.5
1	EMS 5085 mut x Tx623 F3	28.1
2	EMS 5085 mut x Tx623 F3	22.5
3	EMS 5085 mut x Tx623 F3	27.8
4	EMS 5085 mut x Tx623 F3	25.9
5	EMS 5085 mut x Tx623 F3	32.1
6	EMS 5085 mut x Tx623 F3	21.4
7	EMS 5085 mut x Tx623 F3	43.2
8	EMS 5085 mut x Tx623 F3	34.9
9	EMS 5085 mut x Tx623 F3	42.0
10	EMS 5085 mut x Tx623 F3	27.6
11	EMS 5085 mut x Tx623 F3	36.6
12	EMS 5085 mut x Tx623 F3	26.0
13	EMS 5085 mut x Tx623 F3	27.3
14	EMS 5085 mut x Tx623 F3	35.5
15	EMS 5085 mut x Tx623 F3	34.9

Rep	Pedigree	Chlorophyll Meter Reading
16	EMS 5085 mut x Tx623 F3	42.7
17	EMS 5085 mut x Tx623 F3	30.2
18	EMS 5085 mut x Tx623 F3	25.9
19	EMS 5085 mut x Tx623 F3	31.6
20	EMS 5085 mut x Tx623 F3	31.1
21	EMS 5085 mut x Tx623 F3	29.9
22	EMS 5085 mut x Tx623 F3	30.7
23	EMS 5085 mut x Tx623 F3	30.6
24	EMS 5085 mut x Tx623 F3	43.8
25	EMS 5085 mut x Tx623 F3	27.8
26	EMS 5085 mut x Tx623 F3	39.8
27	EMS 5085 mut x Tx623 F3	29.6
28	EMS 5085 mut x Tx623 F3	30.8
29	EMS 5085 mut x Tx623 F3	28.5

12. <u>Stay-Green Experiment Sample 8 Data</u>

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (µs)	Code
1	BTx623	DT	15.1	20.3	2.157	A
2	BTx623	DT	13.8	26.2	2.307	A
3	BTx623	DT	31.0	17.4	2.093	A
4	BTx623	DT	15.4	13.3	1.967	A
5	BTx623	WW	18.2	33.1	2.513	B
6	BTx623	WW	30.2	41.0	2.752	B
7	BTx623	WW	11.1	31.6	2.459	B
8	BTx623	WW	33.5	30.4	2.419	B
9	BTx623	DT	17.7	15.1	2.015	A
10	BTx623	DT	18.5	14.5	1.997	A
11	BTx623	DT	31.6	18.3	2.109	A
12	BTx623	DT	7.7	13.9	1.979	A
13	BTx623	WW	26.8	41.3	2.747	B
14	BTx623	WW	13.4	28.6	2.386	B
15	BTx623	WW	59.3	30.2	2.419	B
16	BTx623	DT	42.2	22.7	2.217	A
17	BTx623	DT	30.8	21.7	2.189	A
18	BTx623	DT	31.4	17.4	2.088	A
19	BTx623	WW	13.6	25.6	2.299	B
20	BTx623	WW	23.2	31.4	2.449	B
21	BTx623	WW	20.0	20.2	2.166	B
22	BTx623	WW	23.9	22.8	2.233	B
23	BTx623	DT	13.6	12.5	1.942	A
24	BTx623	DT	45.7	18.3	2.108	A
25	BTx623	DT	13.7	9.9	1.869	A
26	BTx623	DT	37.6	15.1	2.016	A
27	BTx623	WW	18.8	32.9	2.514	B
28	BTx623	WW	11.5	17.4	2.089	B
29	BTx623	WW	16.2	37.0	2.612	B
1	EMS 932	DT	9.0	20.3	2.157	C
2	EMS 932	DT	10.9	26.2	2.307	C
3	EMS 932	DT	15.3	17.4	2.093	C
4	EMS 932	DT	8.7	13.3	1.967	C
5	EMS 932	WW	16.6	33.1	2.513	D
6	EMS 932	WW	10.3	41.0	2.752	D

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (µs)	Code
7	EMS 932	WW	14.1	31.6	2.459	D
8	EMS 932	WW	10.9	30.4	2.419	D
9	EMS 932	DT	9.7	15.1	2.015	C
10	EMS 932	DT	18.7	14.5	1.997	C
11	EMS 932	DT	8.6	18.3	2.109	C
12	EMS 932	DT	16.8	13.9	1.979	C
13	EMS 932	WW	11.3	41.3	2.747	D
14	EMS 932	WW	14.2	28.6	2.386	D
15	EMS 932	WW	24.1	30.2	2.419	D
16	EMS 932	DT	25.3	22.7	2.217	C
17	EMS 932	DT	21.5	21.7	2.189	C
18	EMS 932	DT	11.6	17.4	2.088	C
19	EMS 932	WW	11.4	25.6	2.299	D
20	EMS 932	WW	9.2	31.4	2.449	D
21	EMS 932	WW	22.5	20.2	2.166	D
22	EMS 932	WW	12.0	22.8	2.233	D
23	EMS 932	DT	11.4	12.5	1.942	C
24	EMS 932	DT	13.9	18.3	2.108	C
25	EMS 932	DT	6.5	9.9	1.869	C
26	EMS 932	DT	9.1	15.1	2.016	C
27	EMS 932	WW	9.2	32.9	2.514	D
28	EMS 932	WW	6.8	17.4	2.089	D
29	EMS 932	WW	5.8	37.0	2.612	D
1	EMS 5085 mut x Tx623 F3	DT	11.5	20.3	2.157	E
2	EMS 5085 mut x Tx623 F3	DT	15.9	26.2	2.307	E
3	EMS 5085 mut x Tx623 F3	DT	32.9	17.4	2.093	E
4	EMS 5085 mut x Tx623 F3	DT	12.5	13.3	1.967	E
5	EMS 5085 mut x Tx623 F3	WW	26.2	33.1	2.513	F
6	EMS 5085 mut x Tx623 F3	WW	33.8	41.0	2.752	F
7	EMS 5085 mut x Tx623 F3	WW	39.7	31.6	2.459	F
8	EMS 5085 mut x Tx623 F3	WW	29.6	30.4	2.419	F
9	EMS 5085 mut x Tx623 F3	DT	10.9	15.1	2.015	E
10	EMS 5085 mut x Tx623 F3	DT	20.0	14.5	1.997	E
11	EMS 5085 mut x Tx623 F3	DT	26.3	18.3	2.109	E
12	EMS 5085 mut x Tx623 F3	DT	9.5	13.9	1.979	E
13	EMS 5085 mut x Tx623 F3	WW	30.5	41.3	2.747	F
14	EMS 5085 mut x Tx623 F3	WW	31.1	28.6	2.386	F

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (μs)	Code
15	EMS 5085 mut x Tx623 F3	WW	35.7	30.2	2.419	F
16	EMS 5085 mut x Tx623 F3	DT	44.5	22.7	2.217	E
17	EMS 5085 mut x Tx623 F3	DT	24.2	21.7	2.189	E
18	EMS 5085 mut x Tx623 F3	DT	20.7	17.4	2.088	E
19	EMS 5085 mut x Tx623 F3	WW	15.9	25.6	2.299	F
20	EMS 5085 mut x Tx623 F3	WW	44.4	31.4	2.449	F
21	EMS 5085 mut x Tx623 F3	WW	17.7	20.2	2.166	F
22	EMS 5085 mut x Tx623 F3	WW	21.3	22.8	2.233	F
23	EMS 5085 mut x Tx623 F3	DT	14.3	12.5	1.942	E
24	EMS 5085 mut x Tx623 F3	DT	36.2	18.3	2.108	E
25	EMS 5085 mut x Tx623 F3	DT	5.8	9.9	1.869	E
26	EMS 5085 mut x Tx623 F3	DT	22.3	15.1	2.016	E
27	EMS 5085 mut x Tx623 F3	WW	21.6	32.9	2.514	F
28	EMS 5085 mut x Tx623 F3	WW	19.0	17.4	2.089	F
29	EMS 5085 mut x Tx623 F3	WW	13.1	37.0	2.612	F

13. <u>Stay-Green Experiment Sample 9 Data</u>

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (μs)	Code
1	BTx623	DT	15.9	13.4	1.962	A
2	BTx623	DT	6.4	8.1	1.806	A
3	BTx623	DT	25.1	12.9	1.949	A
4	BTx623	DT	12.4	10.2	1.875	A
5	BTx623	WW	16.0	14.8	2.019	B
6	BTx623	WW	22.3	33.3	2.491	B
7	BTx623	WW	11.9	16.6	2.064	B
8	BTx623	WW	36.2	20.1	2.157	B
9	BTx623	DT	12.7	13.0	1.959	A
10	BTx623	DT	13.5	12.3	1.927	A
11	BTx623	DT	13.5	11.2	1.902	A
12	BTx623	DT	2.0	11.1	1.899	A
13	BTx623	WW	18.3	26.9	2.336	B
14	BTx623	WW	19.5	15.9	2.056	B
15	BTx623	WW	68.2	27.3	2.348	B
16	BTx623	DT	14.4	14.4	1.990	A
17	BTx623	DT	16.9	12.4	1.934	A
18	BTx623	DT	23.9	9.3	1.839	A
19	BTx623	WW	13.3	13.5	1.972	B
20	BTx623	WW	20.9	22.9	2.231	B
21	BTx623	WW	20.9	12.7	1.956	B
22	BTx623	WW	23.0	18.8	2.132	B
23	BTx623	DT	9.8	12.7	1.943	A
24	BTx623	DT	12.0	11.8	1.923	A
25	BTx623	DT	22.5	10.3	1.884	A
26	BTx623	DT	28.5	10.9	1.900	A
27	BTx623	WW	14.7	26.1	2.322	B
28	BTx623	WW	21.3	25.5	2.317	B
29	BTx623	WW	12.8	31.8	2.466	B
1	EMS 932	DT	8.1	13.4	1.962	C
2	EMS 932	DT	9.4	8.1	1.806	C
3	EMS 932	DT	4.0	12.9	1.949	C
4	EMS 932	DT	2.8	10.2	1.875	C
5	EMS 932	WW	14.7	14.8	2.019	D
6	EMS 932	WW	12.6	33.3	2.491	D
7	EMS 932	WW	9.7	16.6	2.064	D
8	EMS 932	WW	11.9	20.1	2.157	D

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (µs)	Code
9	EMS 932	DT	7.4	13.0	1.959	C
10	EMS 932	DT	7.0	12.3	1.927	C
11	EMS 932	DT	7.8	11.2	1.902	C
12	EMS 932	DT	2.0	11.1	1.899	C
13	EMS 932	WW	10.9	26.9	2.336	D
14	EMS 932	WW	16.8	15.9	2.056	D
15	EMS 932	WW	25.2	27.3	2.348	D
16	EMS 932	DT	16.7	14.4	1.990	C
17	EMS 932	DT	17.4	12.4	1.934	C
18	EMS 932	DT	6.1	9.3	1.839	C
19	EMS 932	WW	12.3	13.5	1.972	D
20	EMS 932	WW	7.4	22.9	2.231	D
21	EMS 932	WW	12.1	12.7	1.956	D
22	EMS 932	WW	12.1	18.8	2.132	D
23	EMS 932	DT	5.8	12.7	1.943	C
24	EMS 932	DT	4.5	11.8	1.923	C
25	EMS 932	DT	10.9	10.3	1.884	C
26	EMS 932	DT	4.0	10.9	1.900	C
27	EMS 932	WW	11.9	26.1	2.322	D
28	EMS 932	WW	10.4	25.5	2.317	D
29	EMS 932	WW	8.4	31.8	2.466	D
1	EMS 5085 mut x Tx623 F3	DT	1.7	13.4	1.962	E
2	EMS 5085 mut x Tx623 F3	DT	16.4	8.1	1.806	E
3	EMS 5085 mut x Tx623 F3	DT	2.0	12.9	1.949	E
4	EMS 5085 mut x Tx623 F3	DT	2.0	10.2	1.875	E
5	EMS 5085 mut x Tx623 F3	WW	23.5	14.8	2.019	F
6	EMS 5085 mut x Tx623 F3	WW	32.1	33.3	2.491	F
7	EMS 5085 mut x Tx623 F3	WW	39.7	16.6	2.064	F
8	EMS 5085 mut x Tx623 F3	WW	36.5	20.1	2.157	F
9	EMS 5085 mut x Tx623 F3	DT	2.0	13.0	1.959	E
10	EMS 5085 mut x Tx623 F3	DT	15.5	12.3	1.927	E
11	EMS 5085 mut x Tx623 F3	DT	18.9	11.2	1.902	E
12	EMS 5085 mut x Tx623 F3	DT	2.0	11.1	1.899	E
13	EMS 5085 mut x Tx623 F3	WW	23.5	26.9	2.336	F
14	EMS 5085 mut x Tx623 F3	WW	37.8	15.9	2.056	F
15	EMS 5085 mut x Tx623 F3	WW	33.0	27.3	2.348	F
16	EMS 5085 mut x Tx623 F3	DT	2.0	14.4	1.990	E
17	EMS 5085 mut x Tx623 F3	DT	16.7	12.4	1.934	E

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (µs)	Code
18	EMS 5085 mut x Tx623 F3	DT	14.5	9.3	1.839	E
19	EMS 5085 mut x Tx623 F3	WW	23.8	13.5	1.972	F
20	EMS 5085 mut x Tx623 F3	WW	29.0	22.9	2.231	F
21	EMS 5085 mut x Tx623 F3	WW	20.5	12.7	1.956	F
22	EMS 5085 mut x Tx623 F3	WW	2.0	18.8	2.132	F
23	EMS 5085 mut x Tx623 F3	DT	10.9	12.7	1.943	E
24	EMS 5085 mut x Tx623 F3	DT	2.0	11.8	1.923	E
25	EMS 5085 mut x Tx623 F3	DT	2.0	10.3	1.884	E
26	EMS 5085 mut x Tx623 F3	DT	2.0	10.9	1.900	E
27	EMS 5085 mut x Tx623 F3	WW	10.8	26.1	2.322	F
28	EMS 5085 mut x Tx623 F3	WW	22.6	25.5	2.317	F
29	EMS 5085 mut x Tx623 F3	WW	8.3	31.8	2.466	F

14. <u>Stay-Green Experiment Sample 10 Data</u>

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (μs)	Code
1	BTx623	DT	25.2	24.9	2.279	A
2	BTx623	DT	7.5	22.6	2.236	A
3	BTx623	DT	13.3	25.3	2.292	A
4	BTx623	DT	11.9	18.1	2.117	A
5	BTx623	WW	5.8	46.7	2.992	B
6	BTx623	WW	13.5	45.2	2.864	B
7	BTx623	WW	21.8	38.9	2.693	B
8	BTx623	WW	19.2	40.7	2.741	B
9	BTx623	DT	11.6	26.5	2.328	A
10	BTx623	DT	30.3	13.8	1.988	A
11	BTx623	DT	7.7	27.4	2.351	A
12	BTx623	DT	2.0	19.9	2.164	A
13	BTx623	WW	21.8	45.9	2.907	B
14	BTx623	WW	8.4	30.2	2.435	B
15	BTx623	WW	58.3	45.3	2.875	B
16	BTx623	DT	8.5	27.8	2.367	A
17	BTx623	DT	19.9	29.5	2.399	A
18	BTx623	DT	35.0	23.9	2.255	A
19	BTx623	WW	9.8	43.7	2.866	B
20	BTx623	WW	10.4	45.1	2.896	B
21	BTx623	WW	10.5	38.7	2.683	B
22	BTx623	WW	22.0	44.8	2.877	B
23	BTx623	DT	12.3	19.1	2.128	A
24	BTx623	DT	2.0	25.2	2.303	A
25	BTx623	DT	9.9	20.9	2.195	A
26	BTx623	DT	3.5	30.7	2.438	A
27	BTx623	WW	6.4	48.7	3.040	B
28	BTx623	WW	14.2	46.5	2.979	B
29	BTx623	WW	7.0	48.6	3.046	B
1	EMS 932	DT	15.4	24.9	2.279	C
2	EMS 932	DT	13.7	22.6	2.236	C
3	EMS 932	DT	3.5	25.3	2.292	C
4	EMS 932	DT	8.7	18.1	2.117	C
5	EMS 932	WW	11.3	46.7	2.992	D
6	EMS 932	WW	12.4	45.2	2.864	D

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (µs)	Code
7	EMS 932	WW	8.3	38.9	2.693	D
8	EMS 932	WW	11.5	40.7	2.741	D
9	EMS 932	DT	18.9	26.5	2.328	C
10	EMS 932	DT	16.2	13.8	1.988	C
11	EMS 932	DT	17.1	27.4	2.351	C
12	EMS 932	DT	2.0	19.9	2.164	C
13	EMS 932	WW	9.9	45.9	2.907	D
14	EMS 932	WW	14.1	30.2	2.435	D
15	EMS 932	WW	17.0	45.3	2.875	D
16	EMS 932	DT	17.5	27.8	2.367	C
17	EMS 932	DT	24.3	29.5	2.399	C
18	EMS 932	DT	15.6	23.9	2.255	C
19	EMS 932	WW	10.9	43.7	2.866	D
20	EMS 932	WW	7.9	45.1	2.896	D
21	EMS 932	WW	12.9	38.7	2.683	D
22	EMS 932	WW	8.7	44.8	2.877	D
23	EMS 932	DT	18.0	19.1	2.128	C
24	EMS 932	DT	16.7	25.2	2.303	C
25	EMS 932	DT	19.5	20.9	2.195	C
26	EMS 932	DT	12.7	30.7	2.438	C
27	EMS 932	WW	8.8	48.7	3.040	D
28	EMS 932	WW	11.6	46.5	2.979	D
29	EMS 932	WW	8.5	48.6	3.046	D
1	EMS 5085 mut x Tx623 F3	DT	2.0	24.9	2.279	E
2	EMS 5085 mut x Tx623 F3	DT	17.9	22.6	2.236	E
3	EMS 5085 mut x Tx623 F3	DT	2.0	25.3	2.292	E
4	EMS 5085 mut x Tx623 F3	DT	2.0	18.1	2.117	E
5	EMS 5085 mut x Tx623 F3	WW	10.9	46.7	2.992	F
6	EMS 5085 mut x Tx623 F3	WW	19.7	45.2	2.864	F
7	EMS 5085 mut x Tx623 F3	WW	25.5	38.9	2.693	F
8	EMS 5085 mut x Tx623 F3	WW	33.5	40.7	2.741	F
9	EMS 5085 mut x Tx623 F3	DT	2.0	26.5	2.328	E
10	EMS 5085 mut x Tx623 F3	DT	29.3	13.8	1.988	E
11	EMS 5085 mut x Tx623 F3	DT	23.1	27.4	2.351	E
12	EMS 5085 mut x Tx623 F3	DT	2.0	19.9	2.164	E
13	EMS 5085 mut x Tx623 F3	WW	15.9	45.9	2.907	F
14	EMS 5085 mut x Tx623 F3	WW	33.9	30.2	2.435	F
15	EMS 5085 mut x Tx623 F3	WW	28.0	45.3	2.875	F

Rep	Pedigree	Water Treatment	Chlorophyll	VWC (%)	PER (μs)	Code
16	EMS 5085 mut x Tx623 F3	DT	2.0	27.8	2.367	E
17	EMS 5085 mut x Tx623 F3	DT	31.1	29.5	2.399	E
18	EMS 5085 mut x Tx623 F3	DT	19.0	23.9	2.255	E
19	EMS 5085 mut x Tx623 F3	WW	16.8	43.7	2.866	F
20	EMS 5085 mut x Tx623 F3	WW	21.7	45.1	2.896	F
21	EMS 5085 mut x Tx623 F3	WW	22.3	38.7	2.683	F
22	EMS 5085 mut x Tx623 F3	WW	2.0	44.8	2.877	F
23	EMS 5085 mut x Tx623 F3	DT	33.0	19.1	2.128	E
24	EMS 5085 mut x Tx623 F3	DT	2.0	25.2	2.303	E
25	EMS 5085 mut x Tx623 F3	DT	2.0	20.9	2.195	E
26	EMS 5085 mut x Tx623 F3	DT	2.0	30.7	2.438	E
27	EMS 5085 mut x Tx623 F3	WW	4.2	48.7	3.040	F
28	EMS 5085 mut x Tx623 F3	WW	5.0	46.5	2.979	F
29	EMS 5085 mut x Tx623 F3	WW	4.2	48.6	3.046	F

15. <u>Stay-Green Experiment Relative Water Content</u>

Rep	Line	Water Treatment	FW (mg)	TW (mg)	DW (mg)	RWC (%)	Code
1	BTx623	DT	4.6	4.5	1.2	103.0	A
1	EMS 932	DT	3.4	3.5	1.0	96.0	B
1	5085 F3	DT	2.6	4.8	1.1	40.5	C
2	BTx623	DT	5.5	6.8	1.1	77.2	A
2	EMS 932	DT	4.1	4.7	1.0	83.8	B
2	5085 F3	DT	3.7	5.9	0.9	56.0	C
3	BTx623	DT	5.7	7.8	1.6	66.1	A
3	EMS 932	DT	3.9	4.3	0.9	88.2	B
3	5085 F3	DT	.	.	.	.	C
4	BTx623	DT	1.3	5.8	1.4	-2.3	A
4	EMS 932	DT	3.6	5.2	0.9	62.8	B
4	5085 F3	DT	.	.	.	.	C
5	BTx623	WW	4.7	5.0	1.4	91.7	D
5	EMS 932	WW	4.7	4.0	1.3	125.9	E
5	5085 F3	WW	4.1	4.5	1.4	87.1	F
6	BTx623	WW	5.7	5.8	1.6	97.6	D
6	EMS 932	WW	3.1	3.6	0.8	82.1	E
6	5085 F3	WW	4.2	4.3	0.9	97.1	F
7	BTx623	WW	5.1	5.3	1.4	94.9	D
7	EMS 932	WW	4.7	4.7	1.1	100.0	E
7	5085 F3	WW	3.9	3.7	0.8	106.9	F
8	BTx623	WW	5.4	5.7	1.4	93.0	D
8	EMS 932	WW	3.8	3.4	0.6	114.3	E
8	5085 F3	WW	4.0	4.5	1.2	84.8	F
9	BTx623	DT	5.0	5.3	1.0	93.0	A
9	EMS 932	DT	3.6	3.6	1.0	100.0	B
9	5085 F3	DT	.	.	.	.	C
10	BTx623	DT	4.6	4.5	1.4	103.2	A
10	EMS 932	DT	3.4	3.5	1.0	96.0	B
10	5085 F3	DT	4.2	4.1	1.0	103.2	C
11	BTx623	DT	4.7	5.3	0.8	86.7	A
11	EMS 932	DT	3.6	3.6	0.8	100.0	B
11	5085 F3	DT	3.9	4.1	1.1	93.3	C
12	BTx623	DT	.	.	.	.	A

Rep	Line	Water Treatment	FW (mg)	TW (mg)	DW (mg)	RWC (%)	Code
12	EMS 932	DT	.	.	.	.	B
12	5085 F3	DT	.	.	.	.	C
13	BTx623	WW	5.3	6.0	1.3	85.1	D
13	EMS 932	WW	4.1	4.2	1.3	96.6	E
13	5085 F3	WW	4.8	4.8	1.3	100.0	F
14	BTx623	WW	4.6	4.8	1.5	93.9	D
14	EMS 932	WW	3.9	4.2	1.1	90.3	E
14	5085 F3	WW	4.5	4.7	1.1	94.4	F
15	BTx623	WW	4.6	4.9	1.0	92.3	D
15	EMS 932	WW	3.6	3.5	0.9	103.8	E
15	5085 F3	WW	4.8	5.0	1.1	94.9	F
16	BTx623	DT	4.8	4.9	1.1	97.4	A
16	EMS 932	DT	4.3	4.7	1.0	89.2	B
16	5085 F3	DT	.	.	.	.	C
17	BTx623	DT	4.7	5.1	0.9	90.5	A
17	EMS 932	DT	3.8	4.0	1.0	93.3	B
17	5085 F3	DT	4.1	4.0	1.6	104.2	C
18	BTx623	DT	5.5	5.0	1.4	113.9	A
18	EMS 932	DT	4.1	3.6	0.9	118.5	B
18	5085 F3	DT	4.1	4.4	1.3	90.3	C
19	BTx623	WW	3.8	4.0	1.4	92.3	D
19	EMS 932	WW	3.5	3.9	1.1	85.7	E
19	5085 F3	WW	4.9	5.0	1.5	97.1	F
20	BTx623	WW	4.7	4.6	1.3	103.0	D
20	EMS 932	WW	3.0	2.9	0.5	104.2	E
20	5085 F3	WW	5.1	5.8	1.6	83.3	F
21	BTx623	WW	4.5	4.9	1.8	87.1	D
21	EMS 932	WW	3.3	3.4	0.9	96.0	E
21	5085 F3	WW	4.0	4.8	0.9	79.5	F
22	BTx623	WW	5.5	5.5	1.6	100.0	D
22	EMS 932	WW	3.9	4.0	1.4	96.2	E
22	5085 F3	WW	.	.	.	.	F
23	BTx623	DT	4.3	4.6	1.3	90.9	A
23	EMS 932	DT	3.4	3.4	1.0	100.0	B
23	5085 F3	DT	4.2	4.4	1.1	93.9	C
24	BTx623	DT	.	.	.	.	A
24	EMS 932	DT	3.7	3.7	0.8	100.0	B

Rep	Line	Water Treatment	FW (mg)	TW (mg)	DW (mg)	RWC (%)	Code
24	5085 F3	DT	.	.	.	.	C
25	BTx623	DT	4.6	4.8	1.1	94.6	A
25	EMS 932	DT	3.4	3.3	1.0	104.3	B
25	5085 F3	DT	.	.	.	.	C
26	BTx623	DT	5.8	6.2	1.6	91.3	A
26	EMS 932	DT	3.3	3.6	0.6	90.0	B
26	5085 F3	DT	.	.	.	.	C
27	BTx623	WW	5.0	5.4	1.6	89.5	D
27	EMS 932	WW	4.1	4.2	1.5	96.3	E
27	5085 F3	WW	3.8	4.1	1.3	89.3	F
28	BTx623	WW	4.3	4.5	1.0	94.3	D
28	EMS 932	WW	4.1	4.0	1.3	103.7	E
28	5085 F3	WW	4.9	5.4	0.9	88.9	F
29	BTx623	WW	5.8	5.6	1.8	105.3	D
29	EMS 932	WW	3.0	3.1	0.7	95.8	E
29	5085 F3	WW	4.7	5.3	1.2	85.4	F

VITA

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Education:

B.S. Agronomy (International Agriculture Minor)   Kansas State University   2010

Work Experience:

2010-2014   Graduate Research Assistantship, Department of Agronomy, Purdue

University

2012   Graduate Teaching Assistant, Department of Agronomy, Purdue

University

2007-2010   Student Office Assistant, Agronomy Teaching Office, Department of

Agronomy, Kansas State University

2009   Summer Intern, Pioneer Hi-Bred, Garden City, KS

2008   Undergraduate Laboratory Assistant, Plant Transformation Laboratory,

            Department of Plant Pathology, Kansas State University

Up to 2010   Hired Hand on Family Farm, Skelton Farms, Larned, KS

Honors and Awards:

2nd Place Graduate Student Poster, Crop Science Society of America Division C-01

            Poster Competition, 2013, Tampa, FL

American Society of Agronomy Golden Opportunity Scholarship, 2009

Kansas Association of Wheat Growers Scholarship, 2008-2010

Charles W. and Lois H. Nauheim Scholarship, 2007-2009

Leslie R. Reinhardt Memorial Scholarship, 2007-2008

Professional Activities:

Taught two sections of Genetics Lab (AGRY 321), tying in concepts learned in the

Genetics lecture course with practical laboratory applications.

As part of a K-12 Outreach program working with fellow plant breeding graduate

students, we presented at an after school program teaching 3rd grade students about the

parts of a cell and how to extract DNA from a strawberry.

Graduate Student Advisor to the Purdue Rodeo Association from 2012-2013.

Recording Secretary for Students of Agronomy, Soils, and Environmental Sciences from

2007-2008.

Graduate Advisor(s):

2013-2014   Dr. Mitchell R. Tuinstra (Department of Agronomy, Purdue University, West Lafayette, IN)

2010-2012   Dr. Herbert W. Ohm (Department of Agronomy, Purdue University, West Lafayette, IN)