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Sorghum:
A Potential Bioenergy Crop

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Enhancing Sustainable Bioenergy Production from Sorghum Through:

Modifying Cell Wall Composition & Increasing Bioconversion and Water-Use Efficiency

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MSc. Internship Report

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DISCLAIMER

This report is based on a four months internship at the agronomy department and genetics institute at the University of Florida, USA, in the period from June 1 to October 1, 2010, as a part of a master degree in Plant Biotechnology from Wageningen University, Netherlands.

SUMMARY OF ACTIVITIES

This report represents most but not all the activities that have been carried out during this period of time. Other activities that have been done and not included in this report include: 1: Selfing, crossing and maintenance (weeds management) of a sorghum nursery at Live Oaks, Florida. 2: Phenotypic field evaluations of Sorghum genotypes based on vigor (mainly height), disease resistance, flowering date, uniformity, as well as measurements of Brix, juice volume, and biomass weight of each genotype and this is at Marianna, Florida. 3: Growing and scoring of *brown midrib* mutants vs. wild type plants of segregating populations of corn and DNA extractions for a Transposons study. 4: Attending some lectures of bioenergy crops course. 5: participating in the bioenergy filed day at Citra, Florida organized by Agronomy department at University of Florida.

ACKNOWLEDGMENT

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A1124: (All x (AY18 x TAM 2566))-24-1-2-1-1
All25: (All x (AY18 x TAM 2566))-25-1-1-1-1
AGS: Photosynthesis rate /stomatal conductance
***bmr*:** brown midrib
***bmr*6:** brown midrib6
***bmr*12:** brown midrib12
***bmr*20**: brown midrib 20
Ci: Intercellular CO2 concentration (µmol CO2 mol-1)
COMT: Caffeic Acid *O*-methyltransferase
Cond: Stomatal conductance to H2O (mol H2O m-2 s-1)
CTAB: Cetyltrimethylammonium Bromide
EH: Early Hegari
EMS: Ethyl Methanesulfonate
FPU: Filter Paper Units
GAX: Glucurono-Arabinoxylans
ICRISAT: International Crops Research Institute for the Semi-Arid-Tropics
KC: Kansas Collier
LSD: Least Significant Difference
Mabe: Mabyana
Mure: Muremba
Photo: Photosynthetic rate (µmol CO2 m-2 s-1)
QTL: Quantitative Trait Loci
ROS: Reactive Oxygen Species
RO: Rox orange
RSA: Root System Architecture
Sacc: Saccaline
SOM: Soil Organic Matter
TE: Transportation Efficiency
TILLING: Targeting Induced Local Lesions in Genomes
Trmmol: Transpiration rate (mmol H2O m-2 s-1)
WT: Wild Type

1. LITERATURE REVIEW

1.1. Plant Abiotic Stress and water scarcity as a major worldwide problem

Environmental stresses, such as drought, salinity, extreme temperatures and radiation represent the most limiting factors on the growth of plants and agricultural production. The set of mentioned stresses, termed as Abiotic Stress, is the main cause of crop loss worldwide (Rodriguez *et al.* 2005). Every year up to 82% of annual crops yield is lost due to abiotic stress and the amount of available productive arable land is continuously decreasing forcing the agricultural production to move to areas where the potential for abiotic stress is even greater (Skinner 2006). Among the abiotic factors, drought is one of the major problems in crop production, preventing plants from realizing their full genetic potential (Boyer 1982). Drought severity depends on different factors, such as moisture storing capacity of soils, evaporative demands and quantity and distribution of rainfall (Wery *et al.* 1994). Drought stress is a major limiting factor to agriculture in arid and semiarid areas and considered as the most important reason of yield reduction in crop plants. Undoubtedly, drought is the most complicated problem because of the large influence of genotype by environment interactions (Leung 2008). The worldwide population is growing exponentially and the demand for water is increasing at an alarming rate, therefore the availability of water is becoming an extremely scarce and there is an increasing demand of water efficient crops.

Globally, there is a vast number of countries (around 80) living with extreme drought conditions, which makes up close to 40% of the world population (Hamdy *et al.* 2003). Around 15% of the worlds irrigated lands produce nearly 30% of the globes food. Due to the rapid growth of the population, the search is on to find new land to be cultivated, however, the most favorable land and resources have already been exploited (Munns 2002). Therefore, it is necessary to generate crop plants that could withstand such harsh conditions.

1.2. Drought stress effects on plants

The effects of drought range from morphological, biochemical and physiological levels are evident at all phenological stages of plant growth at whatever stage the water shortage takes place. Photosynthesis is one of the major metabolic processes that are directly affected by drought. A reduction in photosynthesis results in a decrease in leaf expansion, stomata closure, impaired photosynthetic

machinery enhance formation of reactive oxygen species (ROS), premature leaf senescence, decrease in assimilates translocation and associated reduction in crop production (Farooq *et al.* 2009a). In addition, the stress imposed by drought conditions affects the water relations, such as water-use efficiency, relative water content, leaf water potential, stomatal resistance, transpiration rate, leaf and canopy temperature (Farooq *et al.* 2009b).

1.3. Drought resistance mechanisms on plants

Due to the drought effects on plants, they respond by the induction of several morphological, physiological and molecular mechanisms that enable the plant to withstand the stress. Drought resistance mechanisms can be grouped into three categories, i.e. drought escape, drought avoidance and drought stress tolerance.

Drought escape indicates that plants have adapted by having rapid growth, maturation, flowering/fruiting and senescence, permitting them to reproduce before the environment becomes dry. This keeps tissues from being excessively exposed to dehydration (Price *et al.* 2002).

Drought stress avoidance consists of mechanisms that reduce water loss from plants and improve the water uptake. Reduction of water loss is performed by reducing epidermal (stomatal and lenticular) conductance, thickening of the cuticle (cutin and cuticular waxes) and epicuticular waxes, decreasing absorption of radiation by leaf rolling or folding and reducing evaporation surface (leaf area). Water uptake is improved by the maintenance of turgor through an extensive and efficient (deep and thick) root system with large active surface area and an increase in hydraulic conductance. Plants under drought condition survive by managing a balancing act between maintenance of turgor and reduction of water loss (Mitra 2001).

Drought tolerance is defined as the ability to grow, flower and display economic yield under suboptimal water supply (Farooq *et al.* 2009a). The mechanism of the plant to tolerate the drought stress consists of the maintenance of cellular stability and turgor through osmotic adjustment, compatible solutes, antioxidation and a scavenging defense system (Madhava *et al.* 2006).

1.4. Crop response to drought stress and roots role

Responses to water deficit may occur within a few seconds by changing the phosphorylation status of a protein or within minutes and hours through changing gene expression (Bray 1997). From the agronomic point of view, the ways in which crop plants respond to drought cannot be evaluated without assessing their impact on the productivity. Early studies on the plant response to drought, both on the level of plant growth and crop production, emphasized the adaptability to water deficits on many aspects of shoot growth and functioning including (i) reduced rates of cell division and cell growth which leads to small plant size and reduced leaf area, (ii) earlier maximum grain dry weight and shorter duration of grain filling, (iii) reduced grain yield by reducing the number of tillers, spikes and grains per plant, and (iv) reduction of water loss by either leaf shedding or prolonged stomatal closure which might be undesirable since these two responses also reduce dry matter production (Davies & Zhang 1991; Karamanos & Papatheohari 1999). On the other hand, the development of a deep and extensive root system is a drought adaptation strategy that could efficiently acquire water and nutrients from the deep soil and help the plant meeting evapotranspiration demands.

Root growth and distribution plays an important role in plant response to water availability. They are central in determining the growth and yield of crops in water limited environments and determined by both plant genotype and the soil environment (Robertson *et al.* 1993). Plant roots provide dynamic interface between plants and soil by providing the chlorenchyma cells of stems and leaves with a steady supply of water and dissolved minerals. On the other hand, when the soil dries, a hormone, abscisic acid, is synthesized by the roots and delivered to the shoot where it inhibits leaf expansion and, in some cases, induces stomatal closure before any given change in the water and nutrient status of the leaves (McDonald & Davies 1996). Changing of root morphology under drought stress together with other important traits like yield, leaf area and flowering time are controlled by many genes and are known as quantitative traits (polygenic or complex traits). The regions within the genome that contain those genes are known as Quantitative Trait Loci (QTLs). To facilitate detecting and estimating the effect of these QTLs, and then to utilize them for crop improvement, high density genetic maps (linkage maps) constructed by molecular markers are required. Identifying QTL influencing the response of yield and its components to water deficits aids in our understanding of drought tolerance genetics and helps in the development of more drought tolerant cultivars.

1.5. Sorghum root system

Sorghum has an extensive root system as an adaptation to arid regions with low inherent soil fertility. The root system can penetrate 1.5 to 2.5 m into the soil and extend 1 m away from the stem. In contrast, corn roots typically extend only 0.8 m into the soil and extend 0.5 from the stalk (Pellerin & Pages 1996). This large amount of sorghum root material contributes to the build-up of Soil Organic Matter (SOM) after the harvest of aerial plant parts.

1.6. The world need for bioenergy

There is a significant rise in oil prices due to the increasing demand for fuel globally. Furthermore, it was also reported that at the present rate of energy consumption, petroleum reserves were thought to run out within fifty years, those of natural gas within sixty-five years and coal in approximately 200 years (Soetaert & Vandamme 2006). Therefore, there is a strong need for other alternative renewable resources of energy such as bioethanol. Lignocellulosic biomass as agricultural, industrial and forest residuals accounts for the majority of the total biomass present in the world (Kumar *et al*. 2008). A new second-generation biofuel production process, currently in development, will extract bioethanol from this lignocellulosic biomass; the strengthening substance found in all plant tissues. The lignocellulose is found in large amounts in straw, maize stalks, wood chippings, or other organic materials that are often become available as crop residues. The production of biobased products and bioenergy from less costly renewable lignocellulosic materials is important for the sustainable development (Zhang *et al*. 2006). It is a fact that the material cost of second-generation bioethanol will be far less than first-generation bioethanol and the raw materials can be grown in many more areas of the world. Actually, the second-generation production technology is predicted to more than double bioethanol yields without negatively affecting the food chain, since it allows the alternative fuel to be produced from any organic material. The new second-generation biofeules will also significantly improve energy use efficiency and reduce $C0_2$ emissions compared to first-generation biofeules (Hill 2007).

In second generation biofuel production, the cellulosic and hemicellulosic fractions of biomass are converted into sugars through enzymatic hydrolysis (saccharification), and like in the process of producing ethanol from starch, these sugars are converted into alcohols through fermentation processes (Yuan *et al.* 2008).

1.7. Sorghum as a bioenergy crop

Sorghum (*Sorghum bicolor)* is ranked as the fifth most important grain crop and serves as a major food staple and fodder resource for most of the world, especially in arid and semi-arid regions (Xin *et al.* 2008). Moreover, it is a highly efficient photosynthetic C4 plant species that has been found to be a promising feedstock for bioethanol production and a potential bioenergy feedstock (Carpita & McCann 2008). Sugars derived from the juice, starch from grain, and cellulose and hemicellulosic polysaccharides from biomass of sorghum can be converted to ethanol. Sorghum has the highest water-use efficiency among major crop plants and is unusually tolerant to low soil fertility and traits essential for survival and productivity in arid and semi-arid areas with limited irrigation capability (Xin *et al.* 2008). These traits make sorghum particularly advantageous as an alternative bioenergy feedstock because it can be grown profitably on marginal land and therefore, would not remove more fertile land from existing food and fiber production (Rooney 2004). Moreover, Carpita and McCann (2008) expected maize and sorghum to become key model systems for gene discovery relating to biomass yield and quality in the bioenergy grasses because of genetic investigations and breeding successes achieved in these two species.

Because of its adaptation to suboptimal growing conditions and its potential to produce large amounts of biomass and sugars, sorghum is currently receiving considerable attention as a bioenergy crop that would enable the transition from 'first generation' starch-based ethanol to 'second generation' cellulosic ethanol. This transition is necessary because the use of grain for fuel production is considered unethical (Tenenbaum 2008) and unsustainable. The lack of sustainability is caused by the high demand for nitrogen fertilizer to produce the grain, and nitrogen fertilizer runoff causes ecological problems, drives up the cost of production, and adds greenhouse gasses due to the energy requirements (Eickhout *et al.* 2006; Kim & Dale 2008). Cellulosic ethanol, in contrast, is not produced from grain, but from lignocellulosic biomass (vegetative parts of the plant), which consists primarily of plant cell walls. The cell wall of grasses, such as sorghum, is a complex structure in which cellulose micro-fibrils are embedded in a matrix of hemicellulosic polysaccharides, predominantly glucurono-arabinoxylans (GAX), pectin, cell wall proteins, phenolic compounds, predominantly ferulic acid, p-coumaric acid and lignin (Carpita & Gibeaut 1993). All plant cells have a primary cell wall, but vascular tissue and support tissue also have thick secondary walls which are generally rich in lignin, a complex hydrophobic polymer that plays a significant biological role in providing rigidity, facilitating water transport and offering defense against pests and pathogens. Hence, lignin is integral to plant growth, survival and reproductive function. The lignin monomers – p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol – are

synthesized via the concerted action of the shikimic acid and phenylpropanoid pathways, and polymerize via an oxidative coupling mechanism (Hatfield & Vermerris 2001; Ralph *et al.* 2004).

2. Determination of enzymatic hydrolysis efficiency and Klason lignin content

2.1. Introduction

Lignin is a major constituent of secondary cell walls (Figure 1) (Rose 2003). Between 10-25 % of total plant dry matter is made up of this cell wall component (Sticklen 2008). Cross-linking of lignin with cell wall polysaccharides is not fully understood (Sticklen 2007). lignin is the main barrier to cell wall polysaccharides conversion into fermentable sugars through enzymatic hydrolysis (Sticklen 2008), this is due to its cross links with other cell wall polymers like hemicellulose, which increases recalcitrance of vegetative tissue to hydrolytic enzymes (Dhugga 2007). By decreasing the lignin content, cell walls may become more accessible to hydrolytic enzymes (Chen & Dixon 2007).

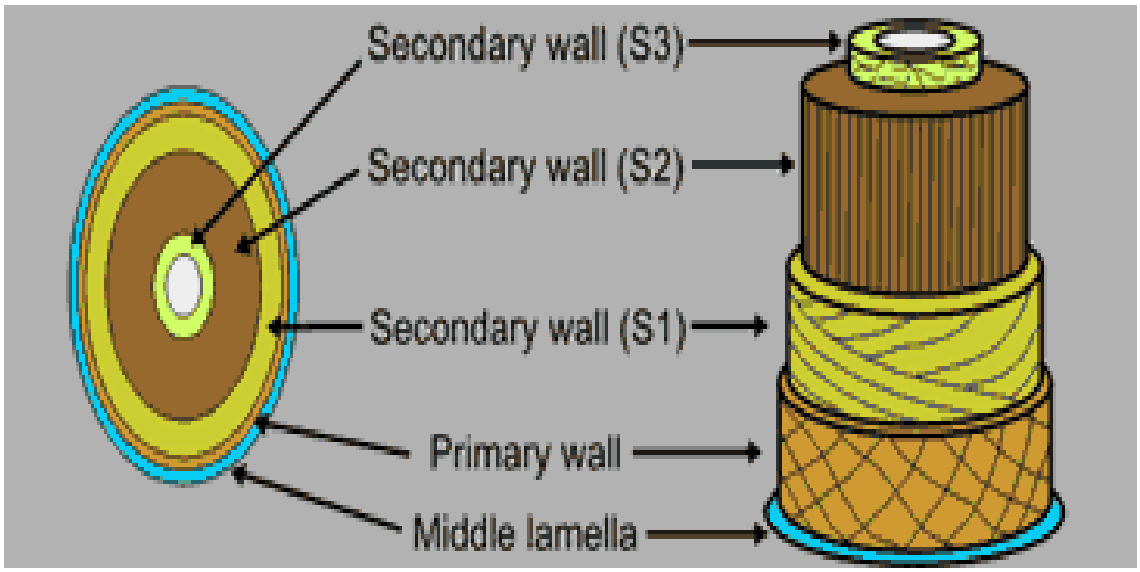

Figure 1. Secondary cell wall structure (Sticklen 2008)

The breakdown of cell walls into its components and releasing the sugars locked up in lignocellulose is technically challenging. It comprises the breakdown of the lignocellulose structure, the release of cellulose sugars by enzymatic hydrolysis (a process often called saccharification) and finally the fermentation of sugars into ethanol (Figure 1). To improve the release of fermentable sugars, a pre-treatment is applied to increase the accessibility of cellulosic polysaccharides to hydrolyzing enzymatic complexes (Sanchez & Cardona 2008). Unluckily, this process increases the total costs of the whole

operation. In fact, the pretreatment costs and cellulases increase the total costs of cellulosic ethanol by a factor of two to three when compared to maize grain ethanol (Sticklen 2008). Somerville (2007), however, stated that "making various steps in the bioethanol production process more efficient will lead to reduction of costs". He expected that cellulosic biofuels will be less expensive than liquid fossil fuel in future. Breeding is considered to be one of the ways that can contribute to lower costs of the processes needed for bioconversion of the cell walls into bioethanol.

2.2. Research Objectives and Questions

The objective of this experiment was; therefore, to investigate the genotypic variation of the different mutants as well as the environmental effects (field, plant, and replicate (block)) effects on enzymatic saccharification parameters, i.e. amount of glucose release from dry matter after enzymatic hydrolysis as an indicator of digestibility and klason lignin concentration in the sorghum stover cell walls.

Research questions:

- Are there differences in bioconversion related traits (glucose content and klason lignin) in sorghum stover due to genotypic differences?
- Which mutants are promising as a lignocellulosic feedstock for bioethanol production?
- Are there differences between genotypes in enzymatic hydrolysis (bioconversion) due to field location or replicate?

2.3. Materials and methods

2.3.1. Plant materials

The plant material used for this analysis comprised samples of Six mutant inbred lines (122, 1107, 1168, 1937, 100 and *bmr*20) generated from the inbred line BTx623 by treatment with the chemical agent Ethyl Methanesulfonate (EMS) resulting in numerous phenotypes with altered morphological and agronomic traits observed from M2 and M3 lines in the field. Two of the mutations identified by TILLING and verified by sequencing were detected in the gene encoding Caffeic Acid *O*-methyltransferase (COMT) in two independent mutant lines (Xin *et al.* 2008). The two mutant lines segregated for the expected *brown midrib* (*bmr*) phenotype, a trait associated with altered lignin content and increased digestibility.

2.3.2. Methods

2.3.2.1. Washing samples for the analysis

Washing the stover is primarily done to remove soluble sugars - they will inflate the initial amount of sugars, and impede hydrolysis. This is besides removing salts, and small molecules (hormones, metabolites especially phenolics) which interferes with hydrolysis. Sorghum stover samples were washed by warmed 50 % ethanol and incubated in waterbath for 30 minutes on a temperature of 65° c then in a sonicator for 15 minutes. The pretreated stover was recuperated by filtering through a Whatman GF/A filter, and dried in 50 C drying oven 48H in cap without lid.

2.3.2.2. Enzymatic Saccharification

Enzymatic hydrolysis was performed to release sugars from cell walls, especially glucose (main base unit of cell walls) in order to determine the maximum extent of possible digestibility. A total of 300 mg dry, extracted sorghum stover was weighed, representing the equivalent of 0.1 g cellulose. Enzymatic saccharification was performed according to Laboratory Analytical Procedure 009 from the National Renewable Energy Laboratory (http://www.eere.energy.gov/biomass/analytical_procedures.html#LAP-009). The stover was suspended in 10 ml 50 mM sodium citrate buffer pH 4.8 containing a 1:1 mixture of Novoxyme 188: Celluclast 1.5 L (Sigma, St. Louis MO, USA) to obtain an enzyme loading of 60 Filter Paper Units (FPU) per gram cellulose. Tetracycline at a final concentration of 20 µg/ml was used to prevent microbial growth. The saccharification reaction was carried out for 4 h in 15-ml polypropylene tubes in a shaker-incubator set at 50°C and shaking at 100 rpm. Glucose concentrations were measured using a calibrated One Touch Ultra Smart blood glucose meter (Roche Diagnostics, Indianapolis, IN, USA), as described by Vermerris *et al.* (2002). Statistical analysis of saccharification results was performed using the statistical package SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Two kinds of blanks were included in each run, the first having all the components used in a saccharification test without plant material and the second without adding the enzyme mix. This allows checking the absence or presence of contamination by any microorganisms.

In total 114 samples from seven Genotypes of different replicates, there are four replicates and three rows each (4 * 3 = 12). In each analysis, at least six controls were added from (BTx623).

2.3.2.3. Determination of Klason lignin concentration

Five washed and dried stover samples from five genotypes were randomly chosen from the first three replicates that have been used in the enzymatic hydrolysis experiments which make a total of twenty-

five samples. The stover was extracted in 50 ml warm (60°C) 80% (v/v) ethanol in a sonicator bath for 30 min to remove soluble sugars and other organic molecules. The stover suspension was filtered through a Whatman glass (GF/A) filter, rinsed with warm 80% (v/v) ethanol, and dried in an oven set at 50°C. Klason lignin was determined by the method of Theander and Westerlund (1986) with the modifications described by Hatfield *et al.* (1994).

2.4. Results

2.4.1. Enzymatic Hydrolysis

The Statistical analysis of the enzymatic saccharification data of the samples from the different genotypes after four hours of hydrolysis showed that the mutant genotype 100 had the highest glucose concentration upon enzymatic hydrolysis (473.83) followed by mutant genotype 1167 (317.75 mg/dl), followed by 1107 genotype and *bmr*20 had the lowest glucose concentrations, even lower than the wild type BTx623.

Table 1 shows that the factor Genotype is the only source of variation significantly affecting glucose concentration upon enzymatic hydrolysis of sorghum stover (p <.001) together with the Block with a less extent (p value = 0.018). In the other hand, there is no significant influence of run or plant factors. Moreover, Analysis of Variance (ANOVA) showed that there is no source (Xin vs. Nebraska) effect on the glucose amounts yielded from the enzymatic saccharification.

Table 1. Summary of *P* values from ANOVAs for saccharification parameters

Source of variation	Glucose concentration
Genotype	<.001***
Run	0.067
Block	0.018*
Plant	0.938

******Indicates the level of significance (means that there is a high significance difference P value <.001)***

Table 2. Means of glucose yields of sorghum stover from the different genotypes after four hours of enzymatic saccharification with the standard deviation and the t-value

Genotype	Glucose concentration (mg/dl)	SD	Pr>|t|
BTx 623	239.24	38.19	
1107	313.50	98.09	**0.0002**
BTx 623	239.24	38.19	

1937	267.50	37.64	**0.0275**
BTx 623	239.24	38.19	
122	262.83	52.81	0.0899
BTx 623	239.24	38.19	
1168	317.75	67.04	**<.0001**
BTx 623	239.24	38.19	
*bmr*20	217.00	25.39	0.0638
BTx 623	239.24	38.19	
100	473.83	55.15	**<.0001**

The probability of the t-value was extrapolated from the Student's t distribution. Probability values in bold face reflect statistically significant (P<0.05) differences.

T test was performed to compare each mutant genotype with the wild type control BTx623. It showed that mutant genotypes 1107, 1937, 1168 and 100 are different from the wild type BTx623 (P p <0.05), while two mutant genotypes (122 and *bmr*20) were not significantly different from the wild type genotype BTx623 (table 2). It is also obvious that mutant genotype 1168 has the highest standard deviation and mutant genotype *bmr*20 has the lowest glucose release. Refer to Appendix A for the complete set of all the enzymatic hydrolysis data.

The LSD showed a grouping of four genotypes both 1168 and 1107 fall in one category, as well as 1937 and 122 in one category (table 3). It also showed a continuous variation of other genotypes (100, BTx623 and *bmr*20).

Table 3. T test (LSD) for glucose concentrations in sorghum stover

Means with the same letter are not significantly different			
t Grouping		Mean	Genotype
	A	473.83	100
	B	317.75	1168
	B	313.50	1107
	C	267.50	1937
	C	262.83	122
D	C	239.24	BTx623
D		217.00	*bmr*20

2.4.2. Klason lignin

The Klason lignin concentration in stover of representatives from each of the four allelic groups was determined (Table 4). It was expected to have significant reductions in Klason lignin concentrations on the allelic groups which were not the case as the wild type genotype BTx623 had the lowest lignin concentration while mutant genotype *bmr*20 has the highest lignin concentration. Moreover, two genotypes (122 and *bmr*20) were significantly different from the wild genotype BTx623 while genotypes 100 and 1107 were not significantly different from it (table 4). Refer to Appendix B for the complete set of all the Klason lignin data.

Table 4. Means of Klason lignin of sorghum stover from the different genotypes with the standard deviation and the t-values

Genotype	Mean klason lignin in mg	SD	Pr>|t|
BTx623	32.00	4.84	
100	40.20	6.14	0.1341
122	45.83	6.37	**0.0390**
*bmr*20	52.80	3.89	**0.0015**
1107	39.20	8.98	0.2651

The probability of the t-value was extrapolated from the Student's t distribution. Probability values in bold face reflect statistically significant (P<0.05) differences

Analysis of variance showed that only Genotype contributed significantly to the variation observed for klason lignin (*p value =* 0.05*).* In the other hand, there is no significant replicate effect neither genotype replicate interaction (*p* value less than 0.005) on klason lignin variation (table 5).

Table 5. Summary of *P* values from ANOVA for Klason lignin in sorghum stover

Source of variation	Klason lignin
Genotype	0.005*
Replicate	0.801
Genotype * Replicate	0.696

**** Indicate a significance effect (p <.01)***

2.5. Discussion

Enzymatic hydrolysis: Due to the fact that replicates (blocks) 1, 2, and 3 were not significantly different but differ from replicate 4 and there is no replicate significant effect, it was decided to run only two sets of replicates in the subsequent runs, randomly chosen from the first 3 replicates to save detergents and solutions.

It is obvious that five allelic mutants (100, 122, 1107, 1168, and 1937) have more enhanced enzymatic hydrolysis efficiency than the wild type genotype (BTx623), which indicates that these mutant genotypes can be promising in bioethanol production if their biomass yield is not affected. Mutant genotype bmr20 was the only mutant genotype to yield less glucose than the wild type which suggests reduced importance.

Klason lignin: It was expected that the mutant genotypes will have a lower lignin than the wild type but the difference is too high and the results of the Klason lignin analysis were not expected especially that *bmr*20 has too much lignin (52.8 mg/dl) compared to the wild type BTx623 (32 mg/dl). Actually, sorghum stover contains between 15 and 25% lignin based on published data. In the other hand, the same genotype (*bmr20*) has the lowest glucose content which explains the negative correlation between the cell wall digestibility represented in the enzymatic hydrolysis and the lignin content in the plant cell walls. This is according to many publications that suggested that lignin is the main barrier to cell wall polysaccharides conversion into fermentable sugars through enzymatic hydrolysis (Sticklen 2008), and this is due to its cross links with other cell wall polymers like hemicellulose, which increases recalcitrance of vegetative tissue to hydrolytic enzymes (Dhugga 2007). It was also stated that The composition of the cell wall heavily influences the efficiency of the hydrolysis process and lignin in particular has been shown to drastically impede cellulolytic enzymes (Chang and Holtzapple 2000; Yang and Wyman 2004) in cereals crops like maize and sorghum. Moreover, it is recommended to redo this experiment to make sure that there were no technical errors in the protocol that may have led to these unexpected results.

3. Drought resistance and water-use efficiency in Sorghum

3.1. Project background

The overall goal of this project is to develop tools to enable the production of sorghum (Sorghum bicolor (L.) Moench) as a multi-purpose crop that can be grown sustainably, especially in countries with hot and dry climates, poor soils, and limited supplies of water and fertilizer – conditions found in many developing countries. Under those conditions, sorghum is one of the few crops that can supply food, feed, fodder, energy and feedstock for biopolymers. However, sorghums have been grown for specific purposes, whereby only limited parts of the plant are used. Selection of favorable traits for specific uses has sometimes led to undesirable side-effects, such as sensitivity to drought. Development of multi-purpose sorghums will, therefore, require the incorporation of useful alleles from other germplasm to compensate for undesirable side effects. This process will be most efficient if genomic regions, and ideally specific alleles of individual genes, have been identified. Multi-purpose sorghums will be even more attractive if value-added products can be produced from them. Hence, in order to achieve our goal, the specific Objective of this project is to study the genetic basis of water-use efficiency as a function of root architecture. ICRISAT's unique and expansive lysimetric system will be used to obtain detailed data on water extraction, transpiration efficiency, and water-use efficiency as a function of root architecture in a diverse set of sorghums. This information will be used along with field observations to measure water-use related traits in a recombinant inbred line populations derived from two parents with contrasting drought responses and root architectures (but similar height and maturity) and map quantitative trait loci associated with drought responses.

The two main questions hers are:

- Are any of the levels (root depth, lignin, sugar, juiciness) determining the net photosynthesis and water-use efficiency?
- Is there any genotype (here it is confounded with the level for all the traits except lignin, as one genotype represents a level of the trait) conferring drought resistance, measured as less reduction in photosynthesis and water-use efficiency (Ags), between the drought and the irrigated treatments?

3.2. Materials and methods

3.2.1. Plant materials

The plant materials used in this study consist of twenty-four genotypes that have a number of contrasting traits like: Lignin (wild type versus *bmr*; *bmr*6 and *bmr*12 are two brown midrib mutations in which the activity of cinnamyl alcohol dehydrogenase2 (Saballos *et al.* 2009; Sattler *et al.* 2009) and caffeic acid *O*-methyltransferase (Bout & Vermerris 2003) are reduced, respectively, root architecture (deep vs. shallow), drought response (resistant vs. susceptible), juiciness (juicy vs. dry), Brix (high vs. low) (table 6).

Table 6. All genotypes used in the study with its different levels and factors.

Genotype	Factor	level
Atlas wt	Lignin	wt
Atlas wt	Lignin	wt
Atlas wt	Lignin	wt
Atlas (*bmr*6)	Lignin	*bmr*6
Atlas (*bmr*6)	Lignin	*bmr*6
Atlas (*bmr*6)	Lignin	*bmr*6
Atlas (*bmr*12)	Lignin	*bmr*12
Atlas (*bmr*12)	Lignin	*bmr*12
Atlas (*bmr*12)	Lignin	*bmr*12
Kansas Collier wt	Lignin	wt
Kansas Collier wt	Lignin	wt
Kansas Collier wt	Lignin	wt
Kansas Collier (*bmr*6)	Lignin	*bmr*6
Kansas Collier (*bmr*6)	Lignin	*bmr*6
Kansas Collier (*bmr*6)	Lignin	*bmr*6
Kansas Collier (*bmr*12)	Lignin	*bmr*12
Kansas Collier (*bmr*12)	Lignin	*bmr*12
Kansas Collier (*bmr*12)	Lignin	*bmr*12
Rox Orange wt	Lignin	wt
Rox Orange wt	Lignin	wt
Rox Orange wt	Lignin	wt
Rox Orange (*bmr*6)	Lignin	*bmr*6
Rox Orange (*bmr*6)	Lignin	*bmr*6
Rox Orange (*bmr*6)	Lignin	*bmr*6
Rox Orange (*bmr*12)	Lignin	*bmr*12
Rox Orange (*bmr*12)	Lignin	*bmr*12
Rox Orange (*bmr*12)	Lignin	*bmr*12
Early Hegari wt	Lignin	wt
Early Hegari wt	Lignin	wt
Early Hegari wt	Lignin	wt

Early Hegari (*bmr*6)	Lignin	*bmr*6
Early Hegari (*bmr*6)	Lignin	*bmr*6
Early Hegari (*bmr*6)	Lignin	*bmr*6
Early Hegari (*bmr*12)	Lignin	*bmr*12
Early Hegari (*bmr*12)	Lignin	*bmr*12
Early Hegari (*bmr*12)	Lignin	*bmr*12
B Tx 631 wt	Lignin	wt
B Tx 631 wt	Lignin	wt
B Tx 631 wt	Lignin	wt
B Tx631 (*bmr*6)	Lignin	*bmr*6
B Tx631 (*bmr*6)	Lignin	*bmr*6
B Tx631 (*bmr*6)	Lignin	*bmr*6
B Tx 631(*bmr*12)	Lignin	*bmr*12
B Tx 631(*bmr*12)	Lignin	*bmr*12
B Tx 631(*bmr*12)	Lignin	*bmr*12
Early Hegari wt	Root architecture	Deep
Early Hegari wt	Root architecture	Deep
Early Hegari wt	Root architecture	Deep
BK7	Root architecture	Shallow
BK7	Root architecture	Shallow
BK7	Root architecture	Shallow
(All x (AY18 x TAM 2566))-25-1-1-1-1-1	Drought score	resistant
(All x (AY18 x TAM 2566))-25-1-1-1-1-1	Drought score	resistant
(All x (AY18 x TAM 2566))-25-1-1-1-1-1	Drought score	resistant
(All x (AY18 x TAM 2566))-24-1-2-1-1	Drought score	susceptible
(All x (AY18 x TAM 2566))-24-1-2-1-1	Drought score	susceptible
(All x (AY18 x TAM 2566))-24-1-2-1-1	Drought score	susceptible
Mabeyana	Juiciness	Juicy
Mabeyana	Juiciness	Juicy
Mabeyana	Juiciness	Juicy
Saccaline	Juiciness	Dry
Saccaline	Juiciness	Dry
Saccaline	Juiciness	Dry
Muremba	Juiciness	Juicy
Muremba	Juiciness	Juicy
Muremba	Juiciness	Juicy
Brandes	Brix	Low
Brandes	Brix	Low
Brandes	Brix	Low
M81E	Brix	High
M81E	Brix	High
M81E	Brix	High

3.2.2. Methods

The twenty-four genotypes were grown in two plots, irrigated and dry. Each plot has two blocks, of the twenty-four genotypes each in three rows. Measurements were based on the middle row. The experiment location was Live Oaks, Florida. Plants were manually grown on 16 June 2010. The dry plot didn't receive any irrigation water during the growing period (rain-fed) while the other plot was receiving standard water regimes along the growing season.

3.2.2.1. Physiological measurements, juiciness and Brix

The Photosynthetic rate (µmol CO2 m-2 s-1) and transpiration rate (mmol H2O m-2 s-1) of the seedling leaf from two plants per genotype were assessed using an LI-6400XT portable photosynthesis system (LI-COR Biosciences) (figure 2). This is besides other physiological parameters like Conductance to H2O (mol H2O m-2 s-1), and Intercellular CO2 concentration (µmol CO2 mol-1). Water-use efficiency and transpiration efficiency were calculated as follows: Water-use efficiency (Ags) = Photosynthesis/conductance to H2O, transpiration efficiency (TE) = Photosynthesis/Transpiration. Moreover, Brix (a measurement of the sugar concentration in the sorghum stover) was measured by Brix meter. Juiciness (amount of juice in the sorghum stover) was measured as well after juicing five plants from each genotype using sugarcane juicer. Juiciness and Brix are being evaluated to assess the relationship between each parameter and the water-use efficiency but the complete data are not available yet since not all genotypes are done yet.

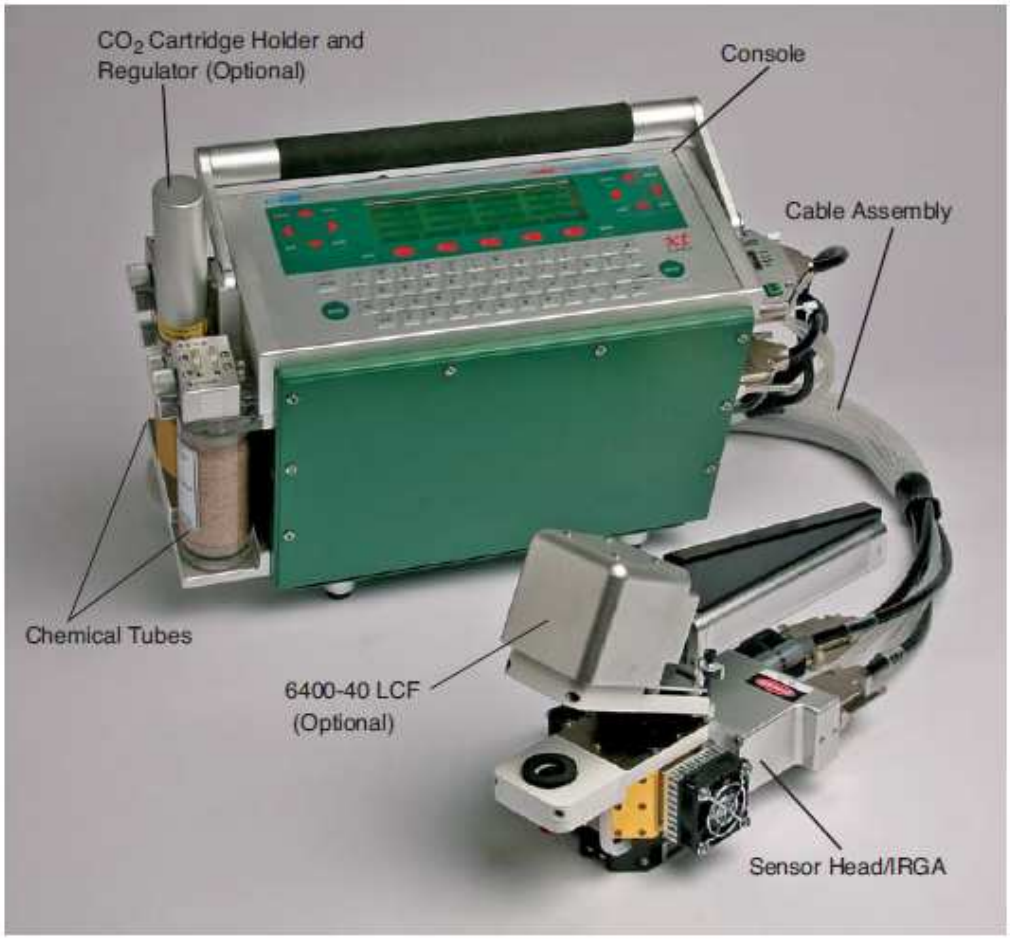

Figure 2. LI-6400XT portable photosynthesis system (LI-COR Biosciences), picture takes from the manual of the LI-6400XT LI-COR, Inc. © Copyright 1998 - 2004, LI-COR, Inc.

3.2.2.2. Biomass dry Weight

The biomass dry weight was based on the two plants that have been used in the physiological measurements after drying them in the oven to get rid of the moisture.

3.2.2.3. Seedling vigor

Seedling vigor was evaluated visually for every genotype and was given a scale from 1 to 10 to see if there is variation in the seedling vigor between the different genotypes being used.

3.2.2.4. Flowering date

Mid-flowering dates of the genotypes from the two plots (the dry and the irrigated one) were monitored. Mid-flowering date is the date when half of the panicles appear (flower) in half of the plants in the row. In cultivated sorghums, the panicle starts developing from 30 to 40 days after germination.

3.2.2.5 DNA extractions

DNA was extracted from the fresh leaves of the twenty four genotypes for genetic analysis by the CTAB method (Winnepenninckx *et al*. 1993). DNA will be used for molecular marker analysis to identify drought tolerance-related QTL.

3.2.2.6 Soil tubes for studying root architecture

The purpose of this experiment is to investigate the genetic basis for root architecture as an important trait for plant drought response as well as investigating the variability between the different genotypes in these traits especially the *bmr* mutants and how they do differ from their wild types. By doing that in soil tubes we hope to get indicators of the behavior and the pattern of the root systems of these plants in the field that would save a lot of time and effort.

3.2.2.6.1. Pilot experiment with black sand

Black sand was thought to be a good medium for the sorghum soil tubes for root characteristics measurements. This is due to two reasons, the first one is that the roots will not bind to the sand particles and would be easy to take out the roots from the tubes and the second reason is that to be able to have a good contrast for taking the pictures of the roots.

3.2.2.6.2. Pilot experiment: sand with 10 % Peat moss

Normal white sand was tried with a 10 % peat moss soil and it did worked and gave a good germination, and this is why it was decided to continue with it.

3.2.2.6.3. Soil tubes with white sand and 10 % peat moss

Twenty-four sorghum genotypes were evaluated for their roots properties. Healthy seeds of each genotype were planted in 100 cm tubes, filled with sand mixed with 10 % peat moss and watered daily. After germination roots lengths development was measured every two days for a period of ten days. A total of 288 tubes were prepared in three patches (replicates), in each patch the twenty four genotypes are represented in four blocks. Genotypes were randomly distributed among blocks. After ten days of root measurements, and when the first roots reached the bottom of the tube, the roots have been

washed and scanned for measuring the root architecture by WinRHIZO (data sown in appendix E without analysis).

3.3. Results

The field experiment showed variation between the dry and the irrigated plot as well as between the twenty four genotypes under study in their response to the drought stress in most of the parameters investigated.

3.3.1 Physiological measurements

***Dry plot*:**

Analysis including *bmr* genotypes showed a continuous variation in respect to Photosynthesis rates in the dry plot with the hybrid genotype All25 having the highest photosynthetic rate (46.26) and genotype BTx631 was having the lowest photosynthetic rate (31.27) (table 7).

Table 7. Means of photosynthesis of wt genotypes with LSD 5% (least significant difference of 5 %)

Means with the same letter are not significantly different				
t Grouping			Mean	Genotype
	B		46.26	All25
	B			
C	B		42.76	Mure
C	B			
C	B		40.47	BK7
C	B			
C	B		40.40	Mabe
C	B			
C	B		39.99	M81E
C	B			
C	B	A	39.99	A1124
C	B	A		

C	B	A	38.99	KC
C	B	A		
C	B	A	38.75	Brandes
C	B	A		
C	B	A	38.71	EH
C	B	A		
C	B	A	38.63	Sacc
C	B	A		
C	B	A	37.36	Atlas
C		A		
C		A	34.15	RO
C		A		
		A	31.27	BTx631
		A		

Analysis of variance of photosynthesis of the dry plot showed that Genotype, block, and level all have a significant effect on photosynthesis as a source of variation (table 8). In the other hand, Analysis of Variance of the water use efficiency (Ags), showed that the only factor that explains the variation is the block effect that was significant (*p* value <.001) (table 8).

Table 8. Summary of *P* values from ANOVA for photosynthesis and water-use efficiency of the dry plot of all genotypes

Source of variation	Photosynthesis	Ags
Genotype	0.033*	0.618
Block	<.001*	<.001*
Level	0.014*	0.193
Genotype. Level	0.017*	0.14

** Indicates a significance effect (p* <.05*)*

Analysis of variance of photosynthesis with only wild type genotypes for the dry plot showed no variation explained by the Genotype factor, as well as the interaction between Genotype and Block. The variation is mainly due to block effect (*p* value = 0.001) (table 10).

Table 10. Summary of *P* values from ANOVA for photosynthesis of the dry plot of only wild type genotypes

Source of variation	Photosynthesis
Genotype	0.806
Block	0.001*
Genotype * Block	0.841

** Indicates a significance effect (p <.05)*

Effects of lignin: Analysis of Variance for photosynthesis showed that the only factor that has an influence big enough to be detectable over the environmental noise is the lignin level (*p* value = 0.029). The interaction between genotype and lignin level almost reach significance (0.072), so we shouldn't discard the genetic background effect on the mutations. The block was the biggest factor as a source of variation (0.018) (table 9). Moreover, Analysis of Variance for water-use efficiency showed that neither the genotype nor the lignin level significantly affect the water use efficiency (*P* value for all of them is less than 0.05). Moreover, Block didn't show a significant source of variation neither its interaction with the genotype (table 9).

Table 9. Summary of *P* values from ANOVA for photosynthesis and water-use efficiency of the dry plot (of *bmr* mutant genotypes)

Source of variation	Photosynthesis	Water-use efficiency
Genotype	0.126	0.66
Lignin Level	0.029*	0.187
Block	0.018*	0.154
Genotype * level	0.072	0.183

** Indicates a significance effect (p <.05)*

LSD test showed no clear grouping. In general, the wild types have significantly higher photosynthetic rates than both *bmr*6 and *bmr*12 in the dry plot, and *bmr*6 is higher than *bmr*12 (table 10).

Table 1010. T Test (LSD) for Photosynthesis (Means with the same letter are not significantly different)

t Grouping	Mean	level
A	39.14	wt
B A	35.48	bmr6
B	33.05	bmr12

Effects of root depth: Table 11 shows that the Block is the only factor that is significantly explains the variation in photosynthetic rates. In the other hand, no variation can be explained by the root depth (*p* value = 0.442). The same thing was noticed for water-use efficiency (table 11).

Table 11. Summary of *P* values from ANOVA for photosynthesis and water-use effciency of the dry plot (taking the root depth as the main factor)

	***P* values**	
Source of variation	**Photosynthesis**	**Water-use efficiency**
Genotype	0.442	0.432
Root depth	0.442	0.432
Block	0.015*	0.03*
Genotype. Block	0.327	0.931

** Indicates a significance effect (p <.05)*

Effects of juiciness: The only factor that significantly affects the photosynthesis and water-use efficiency is the block. Juiciness didn't affect significantly on any of the two parameters (table 12).

Table 12. Summary of *P* values from ANOVA for photosynthesis and water-use efficiency of the dry plot (taking the Juiciness as the main factor)

	***P* values**	
Source of variation	**Photosynthesis**	**Water-use efficiency**
Genotype	0.416	0.981
Juiciness	0.29	0.961
Block	0.026*	0.006*
Genotype * Block	0.567	0.439

** Indicates a significance effect (p <.05)*

Effects of sugar concentration (Brix): None of the factors affected significantly on photosynthesis neither the water-use efficiency (*p* values ≥ 0.05) for all of them (table 13).

Table 13. Summary of *P* values from ANOVA for photosynthesis of the dry plot (taking the sugar concentration (Brix) as the main factor)

		***P* values**
Source of variation	**Photosynthesis**	**Water-use efficiency**
Genotype	0.787	0.588
Sugar(Brix)	0.787	0.622
Block	0.419	0.365
Genotype * Block	0.5	0.699

** Indicates a significance effect (p* <.05*)*

Irrigated plot:

Analysis of variance of photosynthetic rates of the different genotypes in the irrigated plot showed no significant variation due to any of the factors. The only factor that was close to significance was the interaction between Genotype and Level (*p* value = 0.055) (table 14). In the other hand, there is no variation in water-use efficiency between the different genotypes explained by any of the factors analyzed (Genotype, Block, Level, and Genotype X Level interaction).

Table 14. Summary of *P* values from ANOVA for photosynthesis and water-use efficiency of the irrigated plot (taking the means of all Genotypes)

	P values	
Source of variation	photosynthesis	Water-use efficiency
Genotype	0.166	0.073
Block	0.521	0.691
Level	0.399	0.547
Block. Genotype	0.489	0.368
Genotype. Level	0.055	0.999

Average of both dry and irrigated plots:

A correlation study between different physiological measurements and dry weight showed that there is no significant correlation between dry weight and any of the four parameters (Intercellular CO_2 concentration, stomatal conductance, photosynthesis and transpiration (table 15). There was a positive significant correlation between conductance and intercellular co_2 concentration and negative significant correlation between photosynthesis and intercellular co_2 concentration.

Table 1511. Coefficients of correlation and their significance between physiological parameters and dry weight parameters (sample means).

Correlation Coefficients and their significance					
Ci					
Cond	0.676*				
Photo	-0.537*	0.415			
Trmmol	0.284	0.395	0.396		
dry weight	0.137	-0.062	0.098	0.182	
	Ci	Cond	Photo	Trmmol	Dry weight

Means that there is a significant correlation between the different parameters (p <.001)

For Stomatal conductance, photosynthesis/conductance (Ags) and Intercellular CO_2 concentration only the water regime has a significant influence on the variation (*p* value<.001). Actually, the water regime appeared to be a significant source of variation for all the parameters. Block is a significant source of variation for photosynthesis, transpiration, and photosynthesis/transpiration (TE) or transpiration efficiency. The level, in the other hand, was a significant source of variation for photosynthesis only (table 16).

Table 16. Summary of *P* values from ANOVAs for all physiological parameters (means of the two plots)

Source of Variance	*P* values					
	Photosynthesis	**Conductivity**	**Transpiration**	**Ci**	**Ags**	**TE**
Genotype	0.262	0.361	0.753	0.357	0.606	0.495
Block	0.015**	0.238	0.002**	0.311	0.16	0.002**
Level	0.02**	0.101	0.348	0.615	0.38	0.996
Factor	0.154	0.392	0.482	0.582	0.51	0.867
Water Regime	<.001***	<.001***	<.001***	<.001***	<.001***	<.001***

******, **, and * Indicate the level of significance difference *** (p <.01), ** (p = .01), * (P <.05)***

Because of the significant effect of the levels of the traits (*p* value =0.02), it was thought to do another ANOVA to know which levels (root depth, lignin, sugar, juiciness) determining the net photosynthesis as

it was not significant on the other parameters. This is kind of partitioning of the variation. Table 17 shows the table of ANOA for the different levels as sources of variation. The level lignin is the only trait significantly affecting the variance in photosynthesis (*p* value = 0.026). None of the levels significantly determine the water-use efficiency.

Table 17. Summary of *P* values from ANOVAs for photosynthesis and water- use efficiency (Ags) (means of the two plots)

Source of Variance	*P* values	
	Photosynthesis	Ags
Lignin	0.026*	0.142
Root depth	0.982	0.143
Sugar	0.64	0.919
Juiciness	0.409	0.919

**** means significance (P <.05)***

Table 18 shows the values of the different physiological measurements of the different genotypes and shows that Genotype M81E has the highest photosynthetic rate while genotype All25 has the highest conductivity and transpiration rates. In the other hand, genotypes All24, Atlas, and RO has the highest water-use efficiency rates (12.37, 12.25, and 12.11, consequently) as Ags (photosynthesis rate/stomatal conductance) is an indicator of water-use efficiency.

Table 18. Summary of means of the different genotypes across both plots (dry and irrigated)

Genotype	Parameters means					
	Photosynthesis	Conductance	Transpiration	Phot/cond	Ci	Phot/trans
A1124	39.68	0.36	8.35	110.22	121.76	4.87
A1125	47.35	0.54	10.54	87.69	152.12	4.70
Atlas	40.86	0.37	8.35	110.43	122.48	5.04
BK7	42.73	0.53	10.08	80.62	161.48	4.44
Brandes	42.49	0.43	9.29	98.81	128.77	4.87
BTx631	37.81	0.47	9.50	80.45	155.40	4.35
EH	41.87	0.43	9.13	97.37	130.14	4.85

KC	41.41	0.39	8.45	106.18	125.69	5.10
M81E	40.83	0.40	9.12	102.08	134.47	4.72
Mabe	43.10	0.40	9.26	107.75	130.95	4.81
Mure	45.10	0.53	10.38	85.09	148.87	4.69
RO	40.69	0.39	8.96	104.33	123.46	4.85
Sacc	42.33	0.41	9.17	103.24	130.75	4.82

Photosynthetic rate (µmol CO2 m-2 s-1), Ci Intercellular CO2 concentration (µmol CO2 mol-1), Transpiration rate (mmol H2O m-2 s-1), Conductance to H2O (mol H2O m-2 s-1)

Lignin level affected significantly on the different physiological measurements and the wild type genotypes had higher photosynthetic rates than *bmr* mutants as well as higher conductivity, transpiration and Intercellular CO2 concentration. In respect to transpiration efficiency (photosynthesis/transpiration), there is no significance difference between the three levels (wt, *bmr*6, and *bmr*12) of lignin. The opposite was clear in respect to Ags (photosynthesis/conductivity) as *bmr*12 had the highest values followed by *bmr*6 and lastly the wild type genotypes. Moreover, *bmr*6 always had higher values than *bmr*12 in respect to photosynthesis, conductivity, transpiration, and Intercellular CO_2 concentration. Additionally, deep rooted genotypes had higher photosynthetic rates, transpiration efficiency, as well as water-use efficiency ((Ags); photosynthesis/conductivity) than the shallow rooted genotypes which had a higher conductivity, transpiration, and Intercellular CO_2 concentration (table 19).

Juicy genotypes had a higher photosynthesis, conductivity, transpiration, Intercellular CO_2 concentration while dry-stem genotypes have a higher water-use efficiency (Ags) and transpiration efficiency (TE).

Table 19. Summary of physiological measurements (means of the different groups (levels)).

Level	Parameters					
	Photosynthesis	**Conductivity**	**Transpiration**	**Phot/cond (Ags)**	**Intercellular co_2**	**Phot/trans (TE)**
*bmr*12	37.77	0.34	8.03	111.09	120.3	4.91
*bmr*6	40.77	0.42	8.99	97.07	133.83	4.82
Wt	42.61	0.45	9.40	94.69	136.49	4.84
Deep root	44.05	0.47	9.81	93.72	140.41	4.74

Shallow root	42.73	0.53	10.08	80.62	161.48	4.44
Dry	42.33	0.41	9.17	103.24	130.75	4.82
Juicy	44.10	0.46	9.82	95.87	139.91	4.75
High Brix	40.46	0.38	8.81	106.47	126.03	4.82
Low Brix	42.86	0.45	9.60	95.24	137.22	4.78
Resistant	47.35	0.54	10.54	87.69	152.12	4.70
Susceptible	39.68	0.36	8.35	110.22	121.76	4.87

Differences between the two treatments:

Figure 3 shows the differences in photosynthetic rates between of different levels of each traits between the two treatments (drought and irrigated). It shows that the irrigated plot has higher values of photosynthetic rates tan the rain-fed (drought) plot. Moreover, wild type genotypes (wt), deep rooted genotypes, and high sugar (brix) genotypes are the ones that have the least difference between the two treatments.

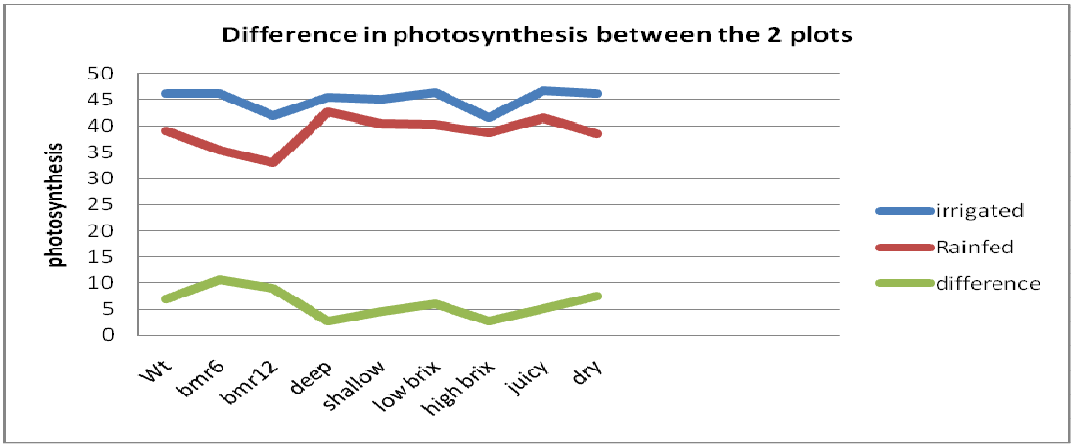

Figure 3. Photosynthetic rates of different levels across both plots and the difference between them

Table 20 gives the measurements of the photosynthetic rates of the individual genotypes confounded with the level of the traits (lignin, root depth, sugar concentration, juiciness) in both plots (drought and irrigated) and the differences between each them. M81E-high brix had lower difference than Brandes-low brix and Muremba-juicy has less difference than the other juicy genotype (Mabeyana-juicy) and the dry-stem one (Saccaline-dry). In the other hand, Ro-wt had the lowest difference followed by Ro-bmr6 and Ro-bmr12 (table 20).

Table 20. Means of photosynthetic rates of the different genotypes in both plots and the differences between them.

Genotypes	Irrigated	Drought	Difference
A11-24-susc	40.4	40	0.4
A11-25-res	48.47	46.27	2.2
Atlas-bmr12	39.96	27.71	12.25
Atlas-bmr6	46.95	45.76	1.19
Atlas-wt	46.24	38.64	7.6
Eh-deep	45.44	42.69	2.75
BK7-Shallow	45.02	40.48	4.54
BTx631-bmr12	39.89	30.49	9.4
BTx631-bmr6	44.77	27.51	17.26
BTx631-wt	44.59	35.43	9.16
EH-bmr12	44.69	37.11	7.58
EHbmr6	45.74	36.8	8.94
Eh-wt	44.41	38.25	6.16
KC-bmr12	39.94	40.04	-0.1
KC-bmr6	45.93	33.81	12.12
KC-wt	45.72	43.12	2.6
Brandes-low	46.26	38.76	7.5
M81E-high	41.44	40.27	1.17
Saccaline-dry	46.07	38.63	7.44
Mabeyana-juicy	45.82	40.4	5.42
Muremba-juicy	47.48	42.76	4.72
RO-bmr12	45.15	28.64	16.51
RO-bmr6	47.01	33.54	13.47
Ro-wt	49.64	40.27	9.37

Figure 4 shows the patterns of the photosynthetic rates of each genotype and the differences of its values between the two treatments. It shows that Atlas-*bmr6* has the least difference in photosynthetic rate between the two treatments (1.19) compared to Atlas-wt and Atlas-*bmr*12. Moreover, Eh-deep, has less difference than BK7-Shallow and BTx631-

*bmr*12 has less difference compared to BTx631-wt and BTx631-*bmr6*. The noticeable genotype was KC-*bmr*12 which had a higher photosynthetic rate in the drought plot than the irrigated one and subsequently less difference than wt and *bmr6*.

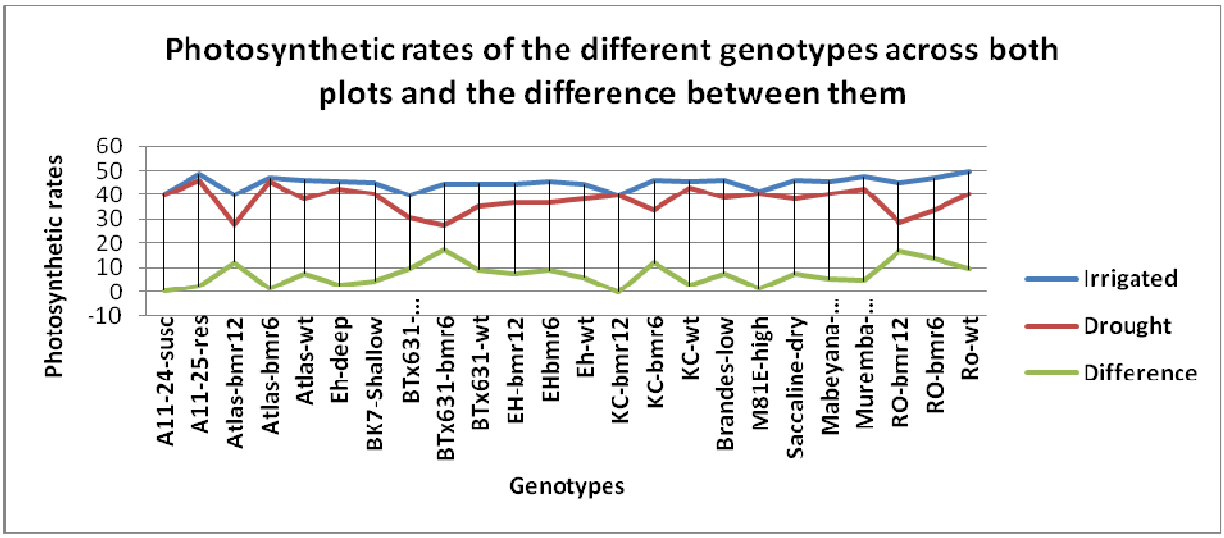

Figure 4. The difference in photosynthetic rates between the irrigated vs. Dry plot of the different genotypes

Water-use efficiency: There was a difference in water-use efficiency between the two plots. Table 21 shows the values of Ags of the different levels in the two plots and the difference between them. Figure 5 as well shows these differences.

Table 21. The values of Ags (water-use efficiency of the different levels of the two plots (irrigated vs. rain-fed and the differences between them.

level	wt	mbr6	bmr12	deep root	shallow root	High brix	Low brix	Juicy	Dry	Resistant	Susceptible
Ags Irrigated	85.77	84.81	107.95	77.69	64.96	90.12	86.67	79.28	95.1	71.28	118.82
Ags Rain-fed	135.35	145.29	147.56	137.63	126.32	138.16	145.53	136.51	136.07	122.36	128.61
Difference	49.58	60.48	39.61	59.94	61.36	48.04	58.86	57.23	40.97	51.08	9.79

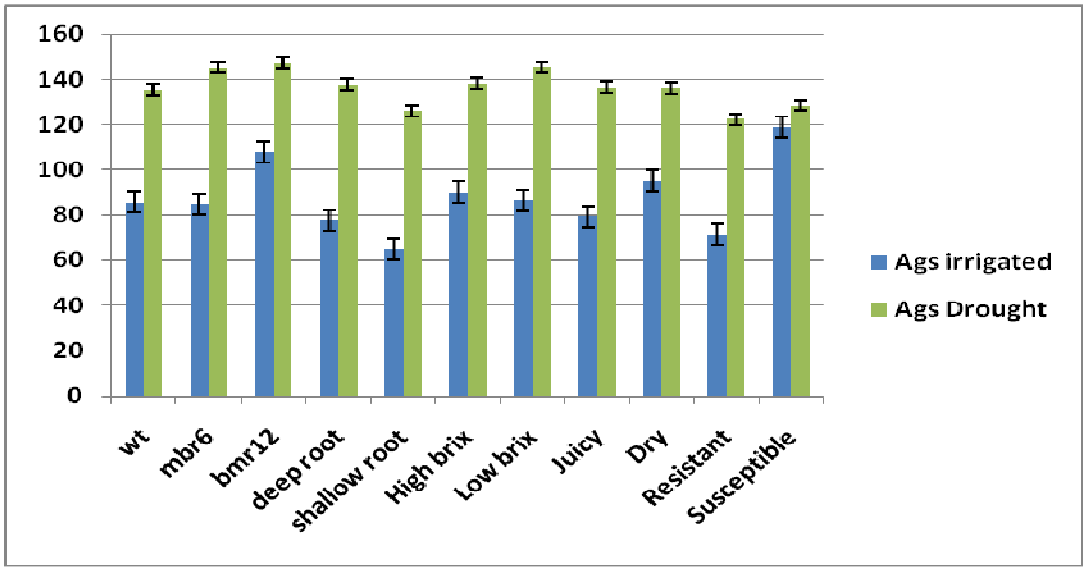

Figure 5. The difference in water-use efficiency between the irrigated vs. Dry plot of the contrasting levels of traits

Figure 6 shows the pattern of the different measurements of water-use efficiency of the different genotypes and differences between the genotypes-levels combinations values in the two treatments. Table 22 shows the exact values of water-use efficiency of each genotype-level combination in both plots (irrigated vs. rain-fed) and the differences between them.

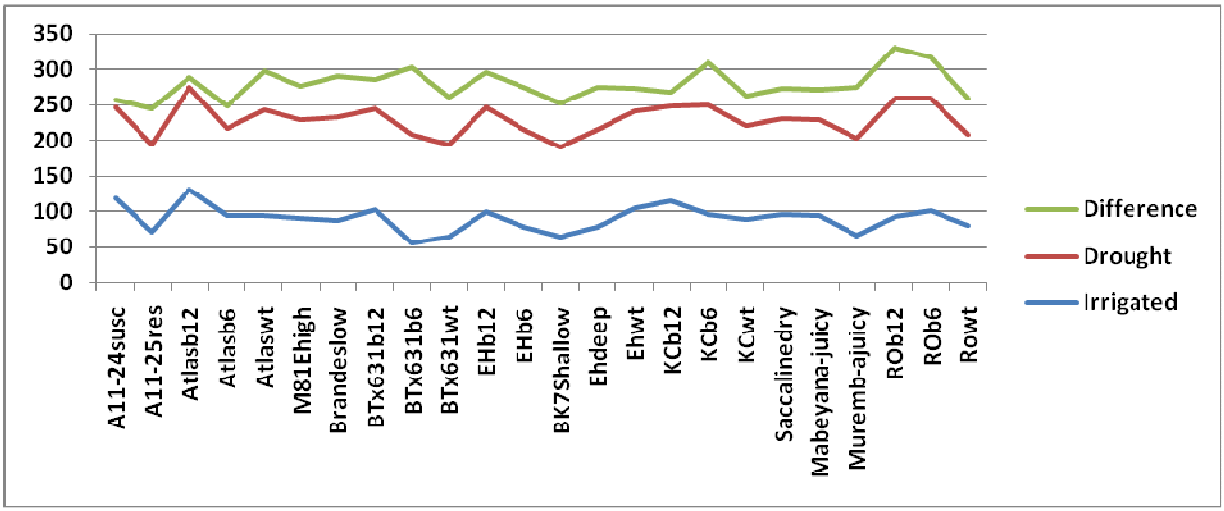

Figure 6. The difference in water-use efficiency between the irrigated vs. Dry plot of the different genotype

Table 22. Means of water use-efficiency of the different genotypes in both plots and the differences between them.

Genotypes	Irrigated	Drought	Difference
A11-25-resistant	118.8	128.6	9.8
A11-24-susceptible	71.28	122.4	51.12
Atlas-bmr12	130.3	144.7	14.4
Atlas-bmr6	93.6	124.1	30.5
Atlas-wt	93.8	148.5	54.7
M81E-high	90.12	138.2	48.08
Brandes-low	86.67	145.5	58.83
BTx631-bmr12	103.1	142.3	39.2
BTx631-bmr6	55.72	151.7	95.98
BTx631-wt	63.78	130.4	66.62
EH-bmr12	99.13	148.3	49.17
EH-bmr6	78.1	137.3	59.2
BK7-Shallow	64.96	126.3	61.34
Eh-deep	77.69	137.6	59.91
Eh-wt	104.1	136.8	32.7
KC-bmr12	114.8	133.9	19.1
KC-bmr6	95.72	154.7	58.98
KC-wt	88.04	131.6	43.56
Saccaline-dry	95.1	136.1	41
Mabeyana-juicy	93.23	135.5	42.27
Muremb-ajuicy	65.32	137.5	72.18
RO-bmr12	92.45	165.9	73.45
RO-bmr6	100.9	158.6	57.7
Ro-wt	79.15	129.4	50.25

3.3.2. Biomass dry weight

Means of dry weight of the dry (rainfed) plot (6.99 gm) was too much lower than the irrigated plot (14.17 gm) which assumes that the water regime affects significantly on the biomass dry weight of the sorghum genotypes.

Analysis of variance showed that the factor Genotype together with the water regime plays an important role as a significant source of variation in dry weight (*p* value < 0.001). Furthermore, the level and the factor play a significance role in explaining the variation but with a less extent (table 23).

Table 2312. Summary of *P* values from ANOVAs for dry weight.

Source of Variance	*p* value
Genotype	< 0.001***
Block	0.092
Level	0.002**
Factor	0.029*
Water Regime	<.001***

******, **, and * Indicate the level of significance difference *** (p <.01), ** (p = .01), * (P <.05)***

There was a variation between the different levels in respect to the dry weight. Mutant genotypes (*bmr*6 and *bmr*12) had a reduced biomass dry weight compared to their wild types counterparts. Moreover, the deep rooted genotypes had a higher dry weight than the shallow one. Juicy genotypes as well had an elevated dry weight compared to the dry genotypes. Furthermore, the resistant genotypes had a significantly higher biomass than the dry ones. In the other hand, sugar content (Brix), didn't affect the dry weight (table 24).

Table 24. Summary of means of dry weights of the different groups (levels).

Level	Dry wt
*bmr*12	8.8
*bmr*6	9.5
Wt	11.9
Deep root	12.8
Shallow root	10.6
Dry	8.8
Juicy	15.5
High Brix	8.7
Low Brix	8.8
Resistant	11.8
Susceptible	8.3

In both of the dry and the irrigated plots, genotypes had variation in their dry weight. Moreover, the ranking of the genotypes did change in both of the plots. Muremba and Mabyana had the highest dry weight in the irrigated plot but in the other hand; they are not the highest in the dry plot. Taking the difference in dry weight of the same genotype in both plots gives an insight of the genotypes that are least affected by the drought stress. Based on that genotype (All25) comes first as least affected then All25, M81E, Sacc, Atlas, Brandes, EH, Btx631, RO, KC, BK7, Mure, and Mabe, consequently (table 25). This also indicates that these genotypes have higher recovery ability than the other genotypes.

Table 25. Means of dry weight of all genotypes from both plots (irrigated and rainfed) and the difference between them.

genotype	A1124	A1125	Atlas	BK7	Brandes	BTx631	EH	KC	M81E	Mabe	Mure	RO	Sacc
irrigated	10	12.39	13.78	15.3	10.58	14.78	14.67	13.39	12.07	21.14	23.5	12.46	12.1
Rainfed	6.78	11.34	8.18	6	4.23	7.19	7.75	5.08	8.33	7.34	10.23	4.77	6.89
difference	3.22	1.05	5.6	9.3	6.35	7.59	6.92	8.31	3.74	13.8	13.27	7.69	5.21

3.2.1. Seedling vigor

Genotype, Block, level, and factor are significant factors that contribute to the variation in seedling vigor (table 26)

Table 2613. Summary of *P* values from ANOVAs for seedling vigor.

Source of Variance	***p* value**
Genotype	< 0.001***
Block	< 0.001***
Level	< 0.001***
Factor	0.004**

******, **, and * Indicate the level of significance difference *** (p <.01), ** (p = .01), * (P <.05)***

There was an obvious difference between the irrigated and the dry plots in respect to seedling vigor. Rating seedling vigor based on a score from 1 to 10. A score of 9 indicates an excellent rate and percent of emergence, an intermediate score of 5 indicates average ratings and a 1 score indicates a very poor seedling vigor and percent of emergence.

Table 27 shows the rates of the seedling vigor of the different genotypes in each plot separately, given a score from 1 to 10 and based on the visual evaluation of the researcher.

Table 27. Rates of seedling vigor of the different genotypes in both plots.

	Seedling vigor score	
	Plot	
Genotype	**Irrigated**	**Rainfed**
(All x (AY18 x TAM 2566))-24-1-2-1-1	5.0	4.5
(All x (AY18 x TAM 2566))-25-1-1-1-1	6.2	7.0
Atlas (*bmr*12)	4.0	5.5
Atlas (*bmr*6)	5.5	6.0
Atlas wt	5.5	5.5
B Tx 631 wt	5.7	5.5
B Tx 631(*bmr*12)	5.5	6.0
B Tx631 (*bmr*6)	5.5	5.5
BK7	3.5	3.5
Brandes	6.5	5.0
Early Hegari (*bmr*12)	7.5	6.0
Early Hegari (*bmr*6)	7.0	5.5
Early Hegari wt	5.5	6.4
Kansas Collier (*bmr*12)	4.0	5.0
Kansas Collier (*bmr*6)	4.5	3.5
Kansas Collier wt	5.2	5.5
M81E	4.5	5.0
Mabeyana	4.5	5.2
Muremba	6.5	6.5
Rox Orange (*bmr*12)	5.5	3.0
Rox Orange (*bmr*6)	5.5	3.5
Rox Orange wt	5.0	4.5
Saccaline	5.0	4.5

3.3.3. Mid flowering date

There were differences in mid flowering date between the different genotypes. Mid-flowering date of the genotypes from both of the plots (the dry and the irrigated one), and genotypes (Roc orange, Kansas Collier, Saccaline, Atlas, early Hegari, B Tx631, BK7, M81E, and Mabeyana), had the earliest mid-flowering dates (table 28).

Table 28. Mid-flowering dates of the early genotypes sorted from early to late.

Row	Genotype	Factor	Level	Mid flowering date
24	Rox Orange (*bmr*6)	Lignin	*bmr*6	09-Aug
153	Rox Orange wt	Lignin	Wt	09-Aug
27	Rox Orange (*bmr*12)	Lignin	*bmr*12	13-Aug
147	Rox Orange (*bmr*6)	Lignin	*bmr*6	13-Aug
150	Rox Orange (*bmr*12)	Lignin	*bmr*12	13-Aug
15	Kansas Collier (*bmr*6)	Lignin	*bmr*6	14-Aug
73	Saccaline	Juiciness	Dry	14-Aug
107	Kansas Collier (*bmr*6)	Lignin	*bmr*6	14-Aug
82	Rox Orange wt	Lignin	Wt	14-Aug
21	Rox Orange wt	Lignin	Wt	15-Aug
104	Kansas Collier wt	Lignin	Wt	15-Aug
12	Kansas Collier wt	Lignin	Wt	16-Aug
116	Atlas (*bmr*6)	Lignin	*bmr*6	16-Aug
113	Atlas wt	Lignin	Wt	17-Aug
3	Atlas wt	Lignin	Wt	18-Aug
6	Atlas (*bmr*6)	Lignin	*bmr*6	18-Aug
122	Early Hegari (*bmr*12)	Lignin	*bmr*12	18-Aug
51	B Tx631 (*bmr*6)	Lignin	*bmr*6	19-Aug
39	Kansas Collier wt	Lignin	Wt	19-Aug
101	Kansas Collier *(bmr*12)	Lignin	*bmr*12	20-Aug
159	B Tx 631 wt	Lignin	Wt	20-Aug
162	B Tx631 (*bmr*6)	Lignin	*bmr*6	20-Aug
61	BK7	Root architecture	Shallow	21-Aug
101	Kansas Collier wt	Lignin	Wt	21-Aug
54	B Tx 631(*bmr*12)	Lignin	*bmr*12	22-Aug
12	M81E	Brix	High	22-Aug
15	Rox Orange wt	Lignin	Wt	22-Aug
88	Rox Orange (*bmr*6)	Lignin	*bmr*6	22-Aug
110	Atlas (*bmr*6)	Lignin	*bmr*6	22-Aug
30	Early Hegari wt	Lignin	Wt	23-Aug
48	B Tx 631 wt	Lignin	Wt	23-Aug
88	Early Hegari wt	Root architecture	Deep	23-Aug
119	Early Hegari wt	Lignin	Wt	23-Aug
134	Saccaline	Juiciness	Dry	23-Aug
33	Kansas Collier (*bmr*12)	Lignin	*bmr*12	23-Aug
85	Rox Orange (*bmr*12)	Lignin	*bmr*12	23-Aug
9	Atlas (*bmr*12)	Lignin	*bmr*12	24-Aug
110	Atlas (*bmr*12)	Lignin	*bmr*12	24-Aug
18	Kansas Collier (*bmr*12)	Lignin	*bmr*12	25-Aug
60	Atlas (*bmr*12)	Lignin	*bmr*12	25-Aug

147	B Tx631 (*bmr*6)	Lignin	*bmr*6	26-Aug
51	B Tx 631 wt	Lignin	Wt	27-Aug
113	Atlas wt	Lignin	Wt	27-Aug
159	Saccaline	Juiciness	Dry	27-Aug
131	Mabeyana	Juiciness	Juicy	28-Aug
150	B Tx 631 wt	Lignin	Wt	28-Aug
70	Mabeyana	Juiciness	Juicy	29-Aug
66	Saccaline	Juiciness	Dry	29-Aug
131	Early Hegari wt	Lignin	Wt	29-Aug

3.3.4. Plants height, juice volume and Brix

Height, volume and sugar content (Brix) data of the different genotypes are not complete because at the end time of the internship not all the genotypes were ready for harvesting. However, table 29 shows some of the measurements that have been taken for the early genotypes of the irrigated plot.

Table 29. Height of the plant, volume of juice and Brix of early Genotypes from the irrigated plot.

Genotype	Level	Block	Height	Volume	Brix
Atlas	Wt	1	241	450	17
Atlas	b6	1	215	365	14.9
Atlas	b12	1	241	440	16
KC	Wt	1	203	250	17.9
KC	b6	1	190	155	16.6
RO	Wt	1	203	200	15.1
RO	b6	1	165	190	17.9
RO	b12	1	177	190	17.1
Sacc	Dry	1	279	170	14.2
KC	b12	2	190	230	14.7
KC	Wt	2	190	265	15
KC	b6	2	127	65	16.5
Atlas	b6	2	190	200	16.1
Sacc	Dry	2	215	125	15.1
RO	b6	2	127	98	19.2
RO	b12	2	127	80	18.6
RO	Wt	2	139	200	18.9

3.3.5. Root characteristics from sorghum soil tubes

As shown in table 30, Rox orange wt had the deepest root system followed by Saccaline wt, and Rox Orange (*bmr*12) in the end of the measurements. In the other hand, Kansas Kollier (*bmr*6) and Muremba

had the least root elongation (table 30). For the complete set of root lengths data refer to Appendix C. Moreover, Appendix D shows the different root characteristics measured by WinRHIZO.

Table 30. Average root length over the 4 blocks in the last measurement sorted from deep to shallow.

Genotype	Average root length in cm ten days post germination
Rox Orange wt	62
Saccaline	60
Rox Orange (*bmr*12)	56
Early Hegari (*bmr*12)	48
Kansas Collier (*bmr*12)	48
Atlas(*bmr*12)	47
Atlas(*bmr*6)	42
BTx 631 wt	41
BK7	40
Kansas Collier wt	40
Brandes	39
BTx 631(*bmr*12)	37
Early Hegari wt	33
Early Hegari (*bmr*6)	33
(All x (Ay18 x TAM 2566))25-1-1-1-1	30
Early Hegari wt deep root	30
Atlas wt	29
Mabeyana	29
(All x (Ay18 x TAM 2566))24-1-1-1-1	29
M81E	29
Rox Orange (*bmr*6)	29
Btx631 (*bmr*6)	28
Muremba	25
Kansas Collier (*bmr*6)	14

3.4. Discussion

The experiment showed a varying response to drought stress of the different genotypes and showed that some of the genotypes that are confounded a good ability to stand drought. The genotypes that had the least difference in photosynthetic rates between the two treatments (irrigated and dry) are the ones that may confer a drought resistance and the same with the water-use efficiency.

Based on that principle in each groups of contrasting genotypes for the different traits we can pick the ones that has the lowest difference and use them as a candidate genotypes that may confer a drought

resistance. This was one of the objectives of the study and was achieved sine we could calculate the differences between the different measurements in both plots.

The second major objective of this study was to investigate if any of the levels (lignin, root-depth, juiciness, and sugar) determines the net photosynthesis rates and the water use efficiency and based on the analysis of variance in the drought plot it was found that the lignin is the only level that affect significantly on the photosynthetic rates but not on water-use efficiency. None of the other levels had a significant effect on the variation. However, the analysis of the different genotypes and groups of genotype-levels combinations revealed differences that may be due to these levels. Actually it was surprising that the root architecture was not significantly affecting on the photosynthesis or the water-use efficiency. However, the deep rooted genotypes had higher values compared to the shallow rooted ones. By completing the root architecture study that was started this will be clear enough.

Late flowering genotypes are favored for bioethanol production, since the prolonged flowering season gives more biomass. Indeed, this is not favored if it was for grain production since, it is preferred to have an early flowering in this case to shorten the season and to escape from diseases.

Ags (photosynthesis/conductance) is used as an indicator for water-use efficiency. The advantage of this parameter is that it doesn't affect by the vapor pressure. Mutant genotypes with altered lignin content (*bmr*6 and *bmr*12) had higher water-use efficiency as well as higher transpiration efficiency (TE) (photosynthesis/transpiration) compared to the wild type in general under drought stress. In the other hand, they had lower dry weight compared to the wild type but not very significant. Besides, *bmr*6 always had higher rates than *bmr*12 in respect to photosynthesis, conductivity, transpiration, and Intercellular CO2 concentration.

Deep rooted genotypes had higher photosynthetic rates, transpiration efficiency, as well as Ags (water-use efficiency; photosynthesis/conductivity) than the shallow rooted genotypes which had a higher conductivity, transpiration, and Intercellular CO_2 concentration. Which indicates the importance of the deep roots as an important morphological traits that the plant needs in the water stress conditions and this is to enable it to get the deep underground water. The opposite is true in the case of shallow rooted plants. Additionally, Juicy genotypes had a higher photosynthesis, conductivity, transpiration, Intercellular CO_2 concentration while dry-stem genotypes have a higher water-use efficiency (Ags) and

transpiration efficiency (TE). It was surprising that the susceptible genotypes to drought stress had a higher Ags, which needs to be confirmed as this parameter is thought to be an indicator of water-use efficiency. But in the other hand, they lower higher photosynthetic rates.

It was not surprising that the *bmr* mutant genotypes (bmr6 and bmr12) had a reduced dry weight compared to the wild types genotypes and the deep rooted, juicy, and drought resistant genotypes had a higher biomass dry weight compared to their contrasting genotypes. Suggesting a more detailed study of these traits and there relation to plant drought responses. Since the main purpose here is feedstock for bioethanol, dry weight is a very important trait to be considered. What is more, taking the difference in dry weight of the different genotypes between the two plots may give an idea about the genotypes that are least affected by drought stress, suggesting a drought resistance mechanism.

Taking the height, Brix, and juice volume of the rest of the genotypes will give us a complete picture of the relationships between these parameters and the drought stress response.

The high block effect that was found suggests a variation in the environmental factors surrounding the experiment, mainly soil variations and may be variations in the irrigation intensity of the pivot used. This finding indicates that these traits are highly affected by environmental factors. This also indicates the complexity of the drought stress because of the large influence of genotype by environment interactions (Leung 2008). Minimizing the block effect could be an important thing to do for a better evaluation of genetic effects.

Sorghum soil tubes: Sorghum seeds didn't grow well in black sand which may be due to the quick dryness or due to the absence of nutrients and this is why was decided to mix sand with 10 % peat moss which did work very well, with a good germination. Despite the fact that the roots characteristics (architecture) were made, the data were not yet analyzed as it should be included with the data from the next two replicates that were not yet done. In the other hand, this method seems to be an efficient method for roots QTL studies for a large number of plants as it is very difficult to do that in the field, but it is still needed to take some sample of some roots from the field to see if they really correlate and the roots from the sorghum grown in tubes are good indicators of the root system of plants grown in the real field.

Finally, the project is still not yet finished and combining the data that will be taken later on will give a better and clearer picture and a broader understanding of the sorghum drought stress responses and its relation to the different factors under study.

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5. Appendices

Appendix A. Combined table of all enzymatic hydrolysis data from all genotypes analyzed.

Sample	Plant	Block	Genotype	Glucose (mg/dl)	Source	Run
1140	1	1	122	240	NE	1
1140	2	1	122	220	NE	1
1140	3	1	122	220	NE	1
1046	1	2	122	286	NE	1
1046	2	2	122	384	NE	1

1046	3	2	122	341	NE	1
1071	1	3	122	220	NE	1
1071	2	3	122	268	NE	1
1071	3	3	122	277	NE	1
1170	1	4	122	245	NE	1
1170	2	4	122	218	NE	1
1170	3	4	122	235	NE	1
1191	1	1	1107	242	NE	1
1192	2	1	1107	238	NE	1
1193	3	1	1107	316	NE	1
1156	1	2	1107	232	NE	1
1156	2	2	1107	200	NE	1
1156	3	2	1107	254	NE	1
1105	1	3	1107	273	NE	1
1105	2	3	1107	380	NE	1
1105	3	3	1107	283	NE	1
1001	1	4	1107	482	NE	1
1001	2	4	1107	364	NE	1
1001	3	4	1107	498	NE	1
1066	2	2	BTx623	203	NE	1
1066	3	2	BTx623	213	NE	1
1034	1	1	BTx623	212	NE	1
1031	2	1	BTx623	237	Xin	1
1031	1	1	BTx623	221	Xin	1
1031	3	1	BTx623	232	Xin	1
1044	1	1	1168	311	NE	1
1044	2	1	1168	301	NE	1
1044	3	1	1168	427	NE	1
1073	1	2	1168	201	NE	1
1073	2	2	1168	298	NE	1
1073	3	2	1168	304	NE	1
1132	1	3	1168	288	NE	1
1132	2	3	1168	300	NE	1
1132	3	3	1168	310	NE	1
1180	1	4	1168	462	NE	1
1180	2	4	1168	288	NE	1
1180	3	4	1168	323	NE	1
1145	1	1	BTx623	303	Xin	2
1145	2	1	BTx623	259	Xin	2
1145	3	1	BTx623	242	Xin	2
1031	1	2	BTx623	202	Xin	2
1031	2	2	BTx623	206	Xin	2

1031	3	2	BTx623	225	Xin	2
1083	1	3	BTx623	183	Xin	2
1083	2	3	BTx623	171	Xin	2
1083	3	3	BTx623	145	Xin	2
1174	1	4	BTx623	247	Xin	2
1174	2	4	BTx623	285	Xin	2
1174	3	4	BTx623	213	Xin	2
1145	1	1	BTx623	290	Xin	3
1145	2	1	BTx623	308	Xin	3
1145	3	1	BTx623	286	Xin	3
1031	1	2	BTx623	233	Xin	3
1031	2	2	BTx623	233	Xin	3
1031	3	2	BTx623	218	Xin	3
1083	1	3	BTx623	224	Xin	3
1083	2	3	BTx623	311	Xin	3
1083	3	3	BTx623	222	Xin	3
1174	1	4	BTx623	234	Xin	3
1174	2	4	BTx623	266	Xin	3
1174	3	4	BTx623	244	Xin	3
1013	1	1	1937	248	NE	1
1013	2	1	1937	227	NE	1
1013	3	1	1937	309	NE	1
1101	1	2	1937	329	NE	1
1101	2	2	1937	325	NE	1
1101	3	2	1937	260	NE	1
1151	1	3	1937	246	NE	1
1151	2	3	1937	216	NE	1
1151	3	3	1937	260	NE	1
1208	1	4	1937	281	NE	1
1208	2	4	1937	232	NE	1
1208	3	4	1937	277	NE	1
1145	1	1	BTx623	300	NE	2
1145	2	1	BTx623	296	NE	2
1145	3	1	BTx623	236	NE	2
1031	1	2	BTx623	227	NE	2
1031	2	2	BTx623	258	NE	2
1031	3	2	BTx623	210	NE	2
1083	1	3	BTx623	212	NE	2
1083	2	3	BTx623	227	NE	2
1083	3	3	BTx623	208	NE	2
1174	1	4	BTx623	260	NE	2
1174	2	4	BTx623	291	NE	2

1174	3	4	BTx623	255	NE	2
1021	1	1	*bmr*20	256	NE	1
1021	2	1	*bmr*20	247	NE	1
1021	3	1	*bmr*20	238	NE	1
1092	1	2	*bmr*20	192	NE	1
1092	2	2	*bmr*20	193	NE	1
1092	3	2	*bmr*20	217	NE	1
1130	1	3	*bmr*20	187	NE	1
1130	2	3	*bmr*20	207	NE	1
1130	3	3	*bmr*20	214	NE	1
1201	1	4	*bmr*20	235	NE	1
1201	2	4	*bmr*20	181	NE	1
1201	3	4	*bmr*20	237	NE	1
1031	1	2	BTx623	351	Xin	4
1031	2	2	BTx623	366	Xin	4
1031	3	2	BTx623	304	Xin	4
1145	1	1	BTx623	545	Xin	4
1145	2	1	BTx623	482	Xin	4
1145	3	1	BTx623	479	Xin	4
1035	1	1	100	385	NE	1
1035	2	1	100	360	NE	1
1035	3	1	100	554	NE	1
1067	1	2	100	314	NE	1
1067	2	2	100	590	NE	1
1067	3	2	100	640	NE	1

Appendix B. Combined data of klason lignin content in all genotypes

Plate number	Sample	Genotype	Block	Filter disc wt	wt before ashing	wt after ashing	klason lignin in mg	Average	SD
1	1035-1	100	1	130	168	133	35	40.2	6.14
2	1035-2	100	1	131	182	133	49		
3	1035-3	100	1	131	179	135	44		
4	1067-1	100	2	132	169	134	35		
5	1067-2	100	2	131	173	135	38		
6	1021-1	*bmr*20	1	131	189	134	55	52.8	3.89

7	1021-3	*bmr*20	1	133	185	139	46		
8	1092-2	*bmr*20	2	134	195	140	55		
9	1092-3	*bmr*20	2	130	192	137	55		
10	1130-1	*bmr*20	3	131	190	137	53		
11	1046-1	122	1	131	178	136	42	45.83	6.37
12	1046-3	122	1	131	193	139	54		
13	1071-1	122	2	132	191	140	51		
14	1071-2	122	2	131	179	140	39		
15	1140-3	122	3	132	183	140	43		
16	1001-1	1107	1	131	181	135	46	39.2	8.98
17	1001-2	1107	1	131	166	134	32		
18	1105-3	1107	2	131	185	136	49		
19	1156-1	1107	3	130	161	133	28		
20	1156-2	1107	3	132	179	138	41		
21	1031-1	btx623xin	1	133	170	134	36	32	4.84
22	1031-2	btx623xin	1	131	168	136	32		
23	1031-3	btx623xin	1	132	160	135	25		
24	1083-1	btx623xin	2	131	177	140	37		
25	1083-3	btx623xin	2	130	168	138	30		

Appendix C. Detailed root length development of patch one of the 24 genotypes.

Tube number	Genotype	Root length in CM						
		Block	M1	M2	M3	M4	M5	M6
1	Brandes	1	18	28	28	34	35	37
2	M81E	1	19.5	28	32	47	48	48
3	Kansas Collier (*bmr*6)	1	18	27	29	33	30	30
4	Btx631 (*bmr*6)	1	0	0	0	0	0	0
5	Kansas Collier wt	1	15	23	29	42	39	42
6	Mabeyana	1	17	19	22	30	28	26
7	Kansas Collier (*bmr*12)	1	23	37	33	20	31	31
8	Atlas(*bmr*6)	1	0	26	23	23	23	20
9	Saccaline	1	21	0	44	44	58	55
10	Atlas(*bmr*12)	1	0	38	7	10	0	0
11	BK7	1	20	37	49	60	57	57
12	(All x (Ay18 x TAM 2566))25-1-1-1-1	1	13	15	10	8	8	6
13	BTx 631(*bmr*12)	1	18	39	50	67	70	67
14	BTx 631 wt	1	17	0	18	20	19	19
15	Early Hegari wt deep root	1	23	45	64	64	64	60
16	Rox Orange (*bmr*12)	1	16	27	30	36	37	41
17	Rox Orange (*bmr*6)	1	21	31	33	54	55	59
18	Early Hegari (*bmr*12)	1	22	22	23	0	23	24
19	Muremba	1	23	40	52	70	71	67
20	Rox Orange wt	1	21	23	20	22	23	18
21	Atlas wt	1	16	23	30	40	34	41
22	Early Hegari wt	1	21	30	35	42	43	44
23	Early Hegari (*bmr*6)	1	20	28	20	53	33	57
24	(All x (Ay18 x TAM 2566))24-1-1-1-1	1	18	19	20	20	20	20
25	(All x (Ay18 x TAM 2566))25-1-1-1-1	2	19	38	48	60	67	67
26	Mabeyana	2	18	37	36	36	36	35
27	Rox Orange wt	2	17	40	38	50	51	54
28	Btx631 (*bmr*6)	2	0	0	0	0	0	0
29	Saccaline	2	15	39	36	41	41	40
30	M81E	2	22	20	18	20	19	19
31	Early Hegari (*bmr*12)	2	22	41	52	53	51	51
32	BK7	2	22	39	52	54	54	55
33	Kansas Collier (*bmr*6)	2	21	41	48	69	66	50
34	Rox Orange (*bmr*12)	2	15	26	29	37	36	36
35	BTx 631 wt	2	10	0	14	13	13	13
36	Early Hegari wt deep root	2	19	30	25	46	49	49
37	Atlas(*bmr*12)	2	14	21	20	23	24	12
38	Kansas Collier wt	2	17	23	23	28	30	30

39	Early Hegari wt	2	0	0	0	19	0	0
40	Rox Orange (*bmr*6)	2	28	42	43	55	66	70
41	Muremba	2	23	41	46	50	46	47
42	Brandes	2	20	38	48	61	65	69
43	(All x (Ay18 x TAM 2566))24-1-1-1-1	2	17	21	28	29	29	30
44	Kansas Collier (*bmr*12)	2	22	38	45	45	47	48
45	Atlas(*bmr*6)	2	14	22	29	31	34	38
46	Early Hegari (*bmr*6)	2	19	32	47	50	54	50
47	BTx 631(*bmr*12)	2	20	27	30	40	42	45
48	Atlas wt	2	15	16	16	0	16	0
49	Kansas Collier wt	3	15	27	30	35	34	34
50	Muremba	3	22	36	42	41	48	48
51	Kansas Collier (*bmr*6)	3	18	27	28	30	29	29
52	Brandes	3	22	23	23	22	21	23
53	Atlas wt	3	18	18	20	25	28	30
54	Btx631 (*bmr*6)	3	0	0	0	0	0	0
55	Early Hegari wt	3	22	30	30	71	71	29
56	Rox Orange (*bmr*6)	3	23	40	53	53	55	60
57	Atlas(*bmr*12)	3	16	20	28	29	30	30
58	BK7	3	23	39	53	72	82	86
59	(All x (Ay18 x TAM 2566))25-1-1-1-1	3	18	18	18	29	28	29
60	Rox Orange (*bmr*12)	3	17	24	32	41	45	47
61	Atlas(*bmr*6)	3	0	0	0	0	8	8
62	M81E	3	19	19	20	22	17	18
63	Rox Orange wt	3	22	30	40	51	49	30
64	BTx 631 wt	3	0	0	0	0	0	0
65	Kansas Collier (*bmr*12)	3	17	28	40	44	40	29
66	Saccaline	3	17	21	31	17	15	29
67	Early Hegari (*bmr*6)	3	19	31	44	56	56	56
68	(All x (Ay18 x TAM 2566))24-1-1-1-1	3	20	32	46	47	44	44
69	Mabeyana	3	20	25	30	35	30	30
70	BTx 631(*bmr*12)	3	19	26	32	34	30	21
71	Early Hegari wt deep root	3	16	17	17	17	13	17
72	Early Hegari (*bmr*12)	3	22	33	50	46	46	47
73	Early Hegari wt deep root	4	25	42	50	66	61	62
74	BTx 631 wt	4	0	0	0	0	0	0
75	Rox Orange (*bmr*12)	4	0	24	30	37	34	32
76	Kansas Collier wt	4	18	27	20	25	26	23
77	BTx 631(*bmr*12)	4	17	19	20	20	18	15
78	Early Hegari (*bmr*12)	4	18	36	56	67	58	87
79	Btx631 (*bmr*6)	4	0	0	0	0	0	0
80	M81E	4	0	0	0	0	0	0
81	Atlas wt	4	20	31	49	47	42	45

82	Saccaline	4	21	30	47	47	45	45
83	Muremba	4	25	36	55	62	65	72
84	Early Hegari (*bmr*6)	4	24	37	50	59	60	63
85	Kansas Collier (*bmr*12)	4	15	22	28	28	27	27
86	Atlas(*bmr*12)	4	19	31	48	50	47	48
87	Early Hegari wt	4	0	0	0	0	0	0
88	Mabeyana	4	23	35	52	41	40	40
89	(All x (Ay18 x TAM 2566))24-1-1-1-1	4	20	33	45	46	43	46
90	Atlas(*bmr*6)	4	16	22	30	35	32	32
91	BK7	4	24	45	60	76	78	87
92	(All x (Ay18 x TAM 2566))25-1-1-1-1	4	14	39	54	66	70	82
93	Rox Orange (*bmr*6)	4	21	32	49	58	55	62
94	Kansas Collier (*bmr*6)	4	20	30	45	64	68	85
95	Rox Orange wt	4	16	19	25	31	41	26
96	Brandes	4	22	34	44	50	47	68

M means measurement

Appendix D. Complete set of physiological data.

water	row	genotype	Factor	Level	Block	plant	Photo	Cond	Ci	Trmmol	VpdL	TE	Ags
rainfed	3	EH	root	Deep	1	1	43.56	0.28	69.39	7.31	2.77	5.96	153.78
rainfed	3	EH	root	Deep	1	2	34.02	0.21	70.56	5.99	3.00	5.68	162.84
rainfed	6	BK7	root	Shallow	1	1	34.37	0.24	101.44	6.67	2.90	5.15	141.67
rainfed	6	BK7	root	Shallow	1	2	32.99	0.21	83.27	6.00	2.94	5.50	154.73
rainfed	9	Brandes	brix	Low	1	2	46.91	0.34	85.81	7.85	2.53	5.97	139.17
rainfed	9	Brandes	brix	Low	1	1	29.72	0.19	80.08	5.21	2.91	5.70	160.23
rainfed	12	M81E	brix	High	1	2	32.68	0.22	89.73	5.88	2.84	5.56	150.74
rainfed	12	M81E	brix	High	1	1	38.76	0.27	90.07	6.65	2.65	5.83	145.41
rainfed	15	RO	lignin	Wt	1	1	24.36	0.17	119.99	4.90	2.92	4.97	140.70
rainfed	15	RO	lignin	Wt	1	2	44.47	0.36	114.65	8.03	2.44	5.54	123.58
rainfed	18	RO	lignin	b6	1	1	38.34	0.26	86.05	6.73	2.75	5.70	147.71
rainfed	18	RO	lignin	b6	1	2	33.73	0.23	93.98	6.12	2.80	5.51	147.35
rainfed	21	RO	lignin	b12	1	1	25.91	0.16	84.77	4.74	3.02	5.46	160.78
rainfed	21	RO	lignin	b12	1	2	23.22	0.14	73.73	4.10	3.07	5.67	170.79
rainfed	24	EH	lignin	b6	1	2	30.17	0.21	103.95	5.50	2.76	5.49	145.37
rainfed	24	EH	lignin	b6	1	1	32.24	0.20	69.41	5.32	2.83	6.05	165.23
rainfed	27	EH	lignin	b12	1	2	26.84	0.16	70.81	4.56	2.96	5.89	169.91
rainfed	27	EH	lignin	b12	1	1	34.29	0.21	69.05	5.54	2.76	6.19	163.66
rainfed	30	EH	lignin	Wt	1	1	42.63	0.31	99.70	7.08	2.43	6.02	135.84
rainfed	30	EH	lignin	Wt	1	2	35.11	0.25	106.96	6.26	2.61	5.61	138.81
rainfed	33	KC	lignin	b12	1	1	40.92	0.36	135.51	7.41	2.27	5.53	115.06
rainfed	33	KC	lignin	b12	1	2	41.31	0.31	105.48	7.21	2.51	5.73	133.56
rainfed	36	KC	lignin	b6	1	1	34.66	0.24	102.93	5.89	2.55	5.88	142.18
rainfed	36	KC	lignin	b6	1	2	35.13	0.24	94.33	6.02	2.65	5.84	146.99
rainfed	39	KC	lignin	Wt	1	1	47.23	0.41	125.65	8.46	2.28	5.58	114.69
rainfed	39	KC	lignin	Wt	1	2	31.54	0.19	63.71	5.02	2.80	6.28	170.16
rainfed	49	BTx631	lignin	b12	1	1	23.69	0.14	84.61	4.24	3.00	5.58	164.56
rainfed	49	BTx631	lignin	b12	1	2	37.29	0.31	133.35	6.91	2.40	5.40	120.12
rainfed	52	BTx631	lignin	Wt	1	2	35.00	0.23	82.02	5.75	2.67	6.09	155.03
rainfed	52	BTx631	lignin	Wt	1	1	21.81	0.12	64.47	3.79	3.15	5.75	179.21
rainfed	55	BTx631	lignin	b6	1	2	24.07	0.17	120.52	4.67	2.83	5.16	141.75
rainfed	55	BTx631	lignin	b6	1	1	34.11	0.22	86.26	5.97	2.80	5.72	153.05
rainfed	58	Atlas	lignin	b12	1	1	24.17	0.14	70.15	4.25	3.10	5.68	173.33
rainfed	58	Atlas	lignin	b12	1	2	9.38	0.07	165.81	2.77	3.69	3.38	125.61
rainfed	61	Atlas	lignin	wt	1	1	37.77	0.24	78.81	6.27	2.69	6.03	154.18
rainfed	61	Atlas	lignin	wt	1	2	36.32	0.23	78.50	6.06	2.73	5.99	155.79
rainfed	64	Atlas	lignin	b6	1	1	49.05	0.42	118.53	8.35	2.23	5.87	117.75
rainfed	64	Atlas	lignin	b6	1	2	47.61	0.39	112.32	8.03	2.29	5.93	123.11
rainfed	67	Saccaline	juice	dry	1	1	35.66	0.24	90.05	6.14	2.70	5.80	149.24
rainfed	67	Saccaline	juice	dry	1	2	32.95	0.20	71.75	5.47	2.82	6.02	163.61

rainfed	70	Muremba	juice	juicy	1	1	36.63	0.22	60.16	5.74	2.74	6.38	167.34
rainfed	70	Muremba	juice	juicy	1	2	47.03	0.34	90.98	7.60	2.41	6.19	137.31
rainfed	73	Mabeyana	juice	juicy	1	1	32.00	0.21	92.46	5.76	2.82	5.56	150.35
rainfed	73	Mabeyana	juice	juicy	1	2	39.59	0.27	90.22	6.87	2.66	5.76	144.08
rainfed	76	A11-24	drought	susc	1	1	43.98	0.34	104.79	7.68	2.47	5.72	130.49
rainfed	76	A11-24	drought	susc	1	2	30.62	0.19	83.63	5.39	2.87	5.68	157.35
rainfed	79	A11-25	drought	res	1	1	50.54	0.43	116.18	8.58	2.21	5.89	116.76
rainfed	79	A11-25	drought	res	1	2	43.40	0.30	85.52	7.24	2.56	6.00	143.27
rainfed	82	RO	lignin	wt	2	1	47.84	0.37	102.05	7.95	2.34	6.01	128.32
rainfed	82	RO	lignin	wt	2	2	44.41	0.35	111.82	8.15	2.50	5.45	125.19
rainfed	85	RO	lignin	b12	2	1	33.20	0.21	74.79	5.87	2.94	5.65	160.00
rainfed	85	RO	lignin	b12	2	2	32.23	0.19	57.84	5.39	2.97	5.98	171.93
rainfed	88	RO	lignin	b6	2	1	28.93	0.16	56.05	4.91	3.07	5.90	176.29
rainfed	88	RO	lignin	b6	2	2	33.16	0.20	70.22	5.74	2.93	5.77	162.98
rainfed	95	KC	lignin	b6	2	1	36.42	0.22	62.87	6.18	2.92	5.89	164.77
rainfed	95	KC	lignin	b6	2	2	29.04	0.18	74.13	5.48	3.20	5.30	164.96
rainfed	98	KC	lignin	b12	2	1	34.11	0.20	55.51	5.82	3.04	5.86	171.89
rainfed	98	KC	lignin	b12	2	2	43.80	0.38	129.87	8.39	2.42	5.22	115.11
rainfed	101	KC	lignin	wt	2	1	49.13	0.43	120.91	8.89	2.33	5.53	115.52
rainfed	101	KC	lignin	wt	2	2	44.59	0.35	111.38	7.81	2.41	5.71	126.16
rainfed	104	EH	root	deep	2	1	47.05	0.47	147.84	9.20	2.22	5.12	100.72
rainfed	104	EH	root	deep	2	2	46.14	0.35	98.04	7.76	2.44	5.94	133.18
rainfed	107	BK7	root	Shallow	2	2	49.16	0.57	167.90	10.38	2.11	4.74	85.86
rainfed	107	BK7	root	Shallow	2	1	45.39	0.37	115.11	8.25	2.45	5.50	123.02
rainfed	110	Atlas	lignin	b6	2	2	45.21	0.38	120.85	8.57	2.48	5.27	119.37
rainfed	110	Atlas	lignin	b6	2	1	41.18	0.30	100.41	7.85	2.78	5.25	136.15
rainfed	113	Atlas	lignin	wt	2	1	44.12	0.33	100.88	7.76	2.54	5.68	133.52
rainfed	113	Atlas	lignin	wt	2	2	36.34	0.24	86.51	6.52	2.84	5.57	150.49
rainfed	116	Atlas	lignin	b12	2	1	43.90	0.35	114.00	7.98	2.48	5.50	125.64
rainfed	116	Atlas	lignin	b12	2	2	33.36	0.22	85.24	6.05	2.92	5.51	154.25
rainfed	119	A11-25	drought	res	2	2	42.30	0.31	95.87	7.48	2.63	5.65	138.55
rainfed	119	A11-25	drought	res	2	1	48.82	0.54	159.71	10.07	2.16	4.85	90.84
rainfed	122	A11-24	drought	susc	2	2	39.69	0.32	123.17	7.73	2.58	5.14	122.89
rainfed	122	A11-24	drought	susc	2	1	45.70	0.44	144.02	9.23	2.34	4.95	103.72
rainfed	125	EH	lignin	b6	2	1	33.54	0.24	109.28	6.21	2.69	5.40	138.22
rainfed	125	EH	lignin	b6	2	2	51.26	0.51	140.85	9.67	2.16	5.30	100.39
rainfed	128	EH	lignin	b12	2	2	43.95	0.37	125.46	8.28	2.43	5.31	117.50
rainfed	128	EH	lignin	b12	2	1	43.36	0.30	87.21	7.37	2.59	5.88	142.30
rainfed	131	EH	lignin	wt	2	1	46.77	0.46	147.08	9.32	2.27	5.02	101.11
rainfed	131	EH	lignin	wt	2	2	28.47	0.17	65.13	5.10	3.15	5.58	171.26
rainfed	141	M81E	brix	high	2	1	49.78	0.47	136.15	9.43	2.24	5.28	104.82
rainfed	141	M81E	brix	high	2	2	39.85	0.26	78.12	6.69	2.70	5.96	151.68
rainfed	144	Brandes	brix	low	2	1	43.11	0.33	104.59	7.55	2.49	5.71	131.63

rainfed	144	Brandes	brix	low	2	2	35.29	0.23	86.38	6.08	2.73	5.80	151.07
rainfed	147	BTx631	lignin	b6	2	1	19.55	0.12	96.32	4.04	3.35	4.84	160.24
rainfed	147	BTx631	lignin	b6	2	2	32.31	0.21	89.77	5.96	2.92	5.42	151.94
rainfed	150	BTx631	lignin	wt	2	1	44.60	0.46	157.29	9.54	2.33	4.68	96.71
rainfed	150	BTx631	lignin	wt	2	2	40.29	0.44	173.77	9.21	2.32	4.38	90.72
rainfed	153	BTx631	lignin	b12	2	1	.	.	.	.	.	.	.
rainfed	153	BTx631	lignin	b12	2	2	.	.	.	.	.	.	.
rainfed	156	Muremba	juice	juicy	2	2	44.78	0.41	137.84	8.91	2.40	5.03	108.83
rainfed	156	Muremba	juice	juicy	2	1	42.61	0.31	97.64	7.61	2.62	5.60	136.47
rainfed	159	Saccaline	juice	dry	2	2	45.64	0.45	147.69	9.38	2.34	4.87	101.70
rainfed	159	Saccaline	juice	dry	2	1	40.27	0.31	111.47	7.78	2.69	5.18	129.75
rainfed	162	Mabeyana	juice	juicy	2	1	46.30	0.34	94.53	8.14	2.57	5.69	134.71
rainfed	162	Mabeyana	juice	juicy	2	2	43.73	0.39	132.61	8.80	2.50	4.97	112.98
irrigated	3	EH	root	deep	1	1	47.22	0.75	205.59	12.39	2.02	3.81	63.32
irrigated	3	EH	root	deep	1	2	42.47	0.48	172.35	10.29	2.43	4.13	88.72
irrigated	6	BK7	root	Shallow	1	1	45.84	0.59	184.26	11.45	2.27	4.00	77.98
irrigated	6	BK7	root	Shallow	1	2	47.95	0.74	202.24	12.50	2.05	3.84	64.66
irrigated	9	Brandes	brix	low	1	1	49.95	0.67	182.44	12.40	2.20	4.03	74.52
irrigated	9	Brandes	brix	low	1	2	36.17	0.28	114.79	8.10	3.10	4.46	130.67
irrigated	12	M81E	brix	high	1	1	48.01	0.66	188.97	12.39	2.22	3.87	72.43
irrigated	12	M81E	brix	high	1	2	41.08	0.47	175.80	10.59	2.54	3.88	87.47
irrigated	15	RO	lignin	wt	1	1	52.17	0.63	167.56	10.63	1.98	4.91	82.59
irrigated	15	RO	lignin	wt	1	2	50.71	0.76	189.54	16.55	2.68	3.06	66.58
irrigated	18	RO	lignin	b6	1	1	46.43	0.55	167.87	14.56	3.06	3.19	84.06
irrigated	18	RO	lignin	b6	1	2	44.84	0.36	112.73	8.92	2.68	5.03	123.88
irrigated	21	RO	lignin	b12	1	1	44.66	0.45	151.94	9.87	2.45	4.53	99.27
irrigated	21	RO	lignin	b12	1	2	47.20	0.67	193.83	11.71	2.09	4.03	70.59
irrigated	24	EH	lignin	b6	1	1	51.20	0.96	214.58	13.43	1.81	3.81	53.57
irrigated	24	EH	lignin	b6	1	2	43.14	0.68	212.07	11.96	2.09	3.61	63.06
irrigated	27	EH	lignin	b12	1	1	34.21	0.24	103.76	7.16	3.07	4.78	139.83
irrigated	27	EH	lignin	b12	1	2	48.83	0.59	170.35	11.47	2.28	4.26	83.24
irrigated	30	EH	lignin	wt	1	1	34.92	0.23	84.80	6.75	3.06	5.17	151.51
irrigated	30	EH	lignin	wt	1	2	51.54	0.82	198.99	12.82	1.95	4.02	63.00
irrigated	33	KC	lignin	b12	1	1	40.31	0.32	116.50	7.48	2.53	5.39	126.73
irrigated	33	KC	lignin	b12	1	2	36.97	0.54	214.70	9.60	2.03	3.85	68.26
irrigated	36	KC	lignin	b6	1	1	47.13	0.49	153.71	9.11	2.11	5.17	96.70
irrigated	36	KC	lignin	b6	1	2	49.08	0.58	170.08	9.53	1.91	5.15	84.74
irrigated	39	KC	lignin	wt	1	1	46.91	0.72	204.57	10.44	1.76	4.49	65.43
irrigated	39	KC	lignin	wt	1	2	42.52	0.30	90.03	6.85	2.44	6.21	141.97
irrigated	49	BTx631	lignin	b12	1	1	46.38	0.69	200.49	12.29	2.13	3.78	67.06
irrigated	49	BTx631	lignin	b12	1	2	35.40	0.25	99.96	7.13	3.00	4.97	141.67
irrigated	52	BTx631	lignin	wt	1	1	44.47	0.50	169.20	10.63	2.40	4.18	88.42
irrigated	52	BTx631	lignin	wt	1	2	46.58	0.91	225.77	13.40	1.87	3.48	50.97

irrigated	55	BTx631	lignin	b6	1	1	42.86	0.64	206.43	11.86	2.19	3.61	66.75
irrigated	55	BTx631	lignin	b6	1	2	46.58	0.85	219.33	13.16	1.95	3.54	55.10
irrigated	58	Atlas	lignin	b12	1	1	38.12	0.24	67.45	6.31	2.77	6.04	160.40
irrigated	58	Atlas	lignin	b12	1	2	43.31	0.34	114.04	8.01	2.51	5.41	125.63
irrigated	61	Atlas	lignin	wt	1	1	45.06	0.36	111.33	8.35	2.53	5.40	125.29
irrigated	61	Atlas	lignin	wt	1	2	43.18	0.45	159.95	9.09	2.28	4.75	96.87
irrigated	64	Atlas	lignin	b6	1	1	46.43	0.44	142.92	8.82	2.22	5.26	104.60
irrigated	64	Atlas	lignin	b6	1	2	47.16	0.49	156.00	9.46	2.17	4.99	95.63
irrigated	67	Saccaline	juice	dry	1	1	47.02	0.48	151.48	10.80	2.52	4.36	97.29
irrigated	67	Saccaline	juice	dry	1	2	47.52	0.65	189.46	12.18	2.22	3.90	73.02
irrigated	70	Muremba	juice	juicy	1	1	47.46	0.83	214.85	13.36	2.01	3.55	57.41
irrigated	70	Muremba	juice	juicy	1	2	45.79	0.64	193.96	12.07	2.23	3.79	71.48
irrigated	73	Mabeyana	juice	juicy	1	1	46.10	0.57	179.65	11.40	2.31	4.04	80.64
irrigated	73	Mabeyana	juice	juicy	1	2	46.22	0.56	175.26	11.36	2.36	4.07	83.19
irrigated	76	A11-24	drought	susc	1	1	44.14	0.49	168.99	10.71	2.45	4.12	89.29
irrigated	76	A11-24	drought	susc	1	2	45.98	0.62	191.20	11.84	2.23	3.88	73.65
irrigated	79	A11-25	drought	res	1	1	52.16	0.74	186.58	12.49	2.05	4.18	70.45
irrigated	79	A11-25	drought	res	1	2	47.42	0.82	214.51	13.01	1.97	3.64	57.72
irrigated	82	RO	lignin	wt	2	1	47.82	0.59	174.55	12.52	2.48	3.82	81.26
irrigated	82	RO	lignin	wt	2	2	47.86	0.56	166.12	12.42	2.58	3.85	86.19
irrigated	85	RO	lignin	b12	2	1	45.51	0.52	168.76	11.86	2.59	3.84	86.96
irrigated	85	RO	lignin	b12	2	2	43.20	0.38	130.85	10.39	2.98	4.16	112.98
irrigated	88	RO	lignin	b6	2	1	49.31	0.49	139.88	11.42	2.66	4.32	101.34
irrigated	88	RO	lignin	b6	2	2	47.48	0.50	153.81	11.82	2.67	4.02	94.29
irrigated	95	KC	lignin	b6	2	1	46.58	0.52	161.08	12.03	2.66	3.87	90.41
irrigated	95	KC	lignin	b6	2	2	40.92	0.37	137.60	10.06	2.98	4.07	111.05
irrigated	98	KC	lignin	b12	2	1	40.29	0.32	114.23	9.56	3.21	4.21	125.89
irrigated	98	KC	lignin	b12	2	2	42.20	0.31	92.64	9.15	3.21	4.61	138.18
irrigated	101	KC	lignin	wt	2	1	44.89	0.74	211.48	13.83	2.28	3.25	60.66
irrigated	101	KC	lignin	wt	2	2	48.56	0.58	168.07	12.69	2.55	3.83	84.08
irrigated	104	EH	root	deep	2	1	46.35	0.81	215.18	14.24	2.18	3.26	57.04
irrigated	104	EH	root	deep	2	2	45.73	0.45	144.47	11.35	2.83	4.03	101.66
irrigated	107	BK7	root	Shallow	2	1	47.76	0.92	222.82	13.90	1.92	3.44	51.64
irrigated	107	BK7	root	Shallow	2	2	38.55	0.59	214.98	11.58	2.29	3.33	65.57
irrigated	110	Atlas	lignin	b6	2	1	48.19	0.72	196.12	13.56	2.29	3.55	67.39
irrigated	110	Atlas	lignin	b6	2	2	46.04	0.43	136.64	11.00	2.84	4.19	106.79
irrigated	113	Atlas	lignin	wt	2	1	47.85	0.63	182.32	12.90	2.43	3.71	76.34
irrigated	113	Atlas	lignin	wt	2	2	48.87	0.64	180.28	12.97	2.41	3.77	76.70
irrigated	116	Atlas	lignin	b12	2	1	34.32	0.23	85.09	7.58	3.48	4.53	151.00
irrigated	116	Atlas	lignin	b12	2	2	44.10	0.52	175.83	11.95	2.61	3.69	84.00
irrigated	119	A11-25	drought	res	2	1	48.25	0.62	178.42	12.91	2.45	3.74	77.97
irrigated	119	A11-25	drought	res	2	2	46.04	0.58	180.28	12.59	2.51	3.66	78.98
irrigated	122	A11-24	drought	susc	2	1	29.06	0.18	72.38	6.09	3.56	4.77	165.22

irrigated	122	A11-24	drought	susc	2	2	38.43	0.26	86.02	8.16	3.30	4.71	147.11
irrigated	125	EH	lignin	b6	2	1	51.71	0.56	150.38	12.50	2.59	4.14	92.64
irrigated	125	EH	lignin	b6	2	2	36.92	0.36	157.31	9.75	2.96	3.79	103.12
irrigated	128	EH	lignin	b12	2	1	46.61	0.61	185.25	12.74	2.44	3.66	76.09
irrigated	128	EH	lignin	b12	2	2	49.10	0.50	147.25	11.95	2.69	4.11	97.35
irrigated	131	EH	lignin	wt	2	1	47.96	0.70	194.85	13.55	2.33	3.54	68.58
irrigated	131	EH	lignin	wt	2	2	43.23	0.32	99.40	9.50	3.15	4.55	133.24
irrigated	141	M81E	brix	high	2	1	37.10	0.28	107.47	8.68	3.32	4.27	133.61
irrigated	141	M81E	brix	high	2	2	39.54	0.59	209.59	12.67	2.50	3.12	66.98
irrigated	144	Brandes	brix	low	2	1	47.91	0.69	192.85	13.41	2.33	3.57	69.49
irrigated	144	Brandes	brix	low	2	2	51.02	0.71	183.39	13.80	2.35	3.70	72.00
irrigated	147	BTx631	lignin	b6	2	1	44.61	0.91	231.49	15.08	2.10	2.96	48.77
irrigated	147	BTx631	lignin	b6	2	2	45.02	0.86	224.90	14.82	2.16	3.04	52.25
irrigated	150	BTx631	lignin	wt	2	1	42.63	0.74	219.80	14.12	2.32	3.02	57.77
irrigated	150	BTx631	lignin	wt	2	2	44.68	0.77	216.32	14.44	2.30	3.09	57.97
irrigated	153	BTx631	lignin	b12	2	1	35.73	0.32	146.56	9.52	3.16	3.75	110.36
irrigated	153	BTx631	lignin	b12	2	2	42.07	0.45	163.54	11.28	2.80	3.73	93.52
irrigated	156	Muremba	juice	juicy	2	1	47.80	0.76	203.60	13.99	2.26	3.42	63.16
irrigated	156	Muremba	juice	juicy	2	2	48.89	0.71	192.08	13.77	2.35	3.55	69.23
irrigated	159	Saccaline	juice	dry	2	1	46.34	0.60	184.07	12.46	2.43	3.72	77.20
irrigated	159	Saccaline	juice	dry	2	2	43.41	0.33	100.14	9.24	3.05	4.70	132.89
irrigated	162	Mabeyana	juice	juicy	2	1	44.91	0.39	125.22	10.27	2.90	4.37	115.35
irrigated	162	Mabeyana	juice	juicy	2	2	46.07	0.49	157.78	11.51	2.65	4.00	93.75

Appendix E. Different root characteristics measured by WinRHIZO from the first patch root measurements.

Tube number	Geno-type	block	Length (cm)	ProjArea (cm2)	SurfArea (cm2)	AvgDiam(mm)	LenPerVol(cm/m3)	RootVolume(cm3)	Tips	Forks	Crossings	0.00<.L.<=0.50	0.50<.L.<=1.00	1.00<.L.<=1.50	1.50<.L.<=2.00
tube 13	BTx 631(*bmr*12)	1	735.05	16.99	53.37	0.23	735.05	0.31	2230.00	3621.00	1008.00	654.64	74.29	5.89	0.21
tube 17	Rox Orange (*bmr*6)	1	486.34	22.28	69.98	0.46	486.34	0.80	1376.00	2957.00	412.00	343.39	105.80	18.68	7.27
tube 31	Early Hegari (*bmr*12)	2	476.10	12.58	39.52	0.26	476.10	0.26	2582.00	2482.00	489.00	420.08	50.05	5.15	0.68
tube 20	Rox Orange wt	1	49.09	1.56	4.90	0.32	49.09	0.04	201.00	129.00	12.00	42.34	6.36	0.35	0.03
tube 56	Rox Orange (*bmr*6)	3	1195.96	36.73	115.39	0.31	1195.96	0.89	2699.00	6458.00	1523.00	1019.89	143.29	21.09	6.22
tube 51	Kansas Collier (*bmr*6)	3	115.35	3.04	9.56	0.26	115.35	0.06	400.00	594.00	104.00	103.66	9.20	2.47	0.02
tube 95	Rox Orange wt	4	206.60	6.02	18.90	0.29	206.60	0.14	756.00	1466.00	287.00	177.52	23.78	4.70	0.51
tube 24	(All x (Ay18 x TAM 2566))24-1-1-1-1	1	149.47	4.98	15.64	0.33	149.47	0.13	362.00	1189.00	207.00	127.73	12.24	5.69	2.51
tube 21	Atlas wt	1	224.30	6.19	19.43	0.28	224.30	0.13	953.00	1413.00	250.00	198.30	22.08	3.27	0.61
tube 16	Rox Orange (*bmr*12)	1	218.13	5.64	17.71	0.26	218.13	0.11	849.00	892.00	164.00	198.19	15.89	3.11	0.64
tube 27	Rox Orange wt	2	369.37	10.80	33.92	0.29	369.37	0.25	962.00	1949.00	361.00	319.57	39.07	7.05	2.19
tube 49	Kansas Collier wt	3	321.74	7.86	24.70	0.24	321.74	0.15	727.00	1710.00	417.00	297.08	20.85	3.43	0.39
tube 30	M81E	2	156.58	4.29	13.49	0.27	156.58	0.09	420.00	1114.00	257.00	134.03	19.00	3.33	0.22
tube 88	Mabeyana	4	282.89	8.35	26.24	0.30	282.89	0.19	999.00	1864.00	388.00	237.63	35.78	7.93	1.44
tube 85	Kansas Collier (*bmr*12)	4	83.80	2.57	8.08	0.31	83.80	0.06	325.00	670.00	147.00	67.40	12.48	2.95	0.82
tube 36	Early Hegari wt deep root	2	330.19	11.50	36.12	0.35	330.19	0.32	1031.00	2451.00	386.00	271.13	42.66	11.00	2.57
tube 52	Brandes	3	241.65	7.62	23.95	0.32	241.65	0.19	763.00	1642.00	250.00	194.81	39.96	5.46	0.83
tube 60	Rox Orange (*bmr*12)	3	240.13	8.83	27.74	0.37	240.13	0.26	865.00	2244.00	344.00	184.75	35.87	12.56	5.36
tube 59	(All x (Ay18 x TAM 2566))25	3	206.88	6.83	21.45	0.33	206.88	0.18	658.00	1684.00	298.00	167.08	30.49	6.78	2.31

	-1-1-1-1														
tube 53	Atlas wt	3	119.36	3.00	9.41	0.25	119.36	0.06	393.00	760.00	130.00	108.90	7.76	2.16	0.48
tube 55	Early Hegari wt	3	509.06	14.93	46.89	0.29	509.06	0.34	1847.00	3190.00	593.00	429.63	68.05	9.50	1.12
tube 47	BTx 631(*bmr* 12)	2	241.49	7.12	22.35	0.29	241.49	0.17	806.00	1253.00	227.00	209.07	27.96	3.85	0.56
tube 57	Atlas (*bmr*12)	3	200.33	6.69	21.00	0.33	200.33	0.18	607.00	1825.00	334.00	158.60	30.49	8.42	2.17
tube 48	Atlas wt	2	82.30	2.04	6.41	0.25	82.30	0.04	277.00	349.00	92.00	74.94	6.57	0.74	0.06
tube 91	BK7	4	842.39	33.16	104.19	0.39	842.39	1.03	3218.00	8745.00	1820.00	664.74	92.16	47.84	20.99
tube 84	Early Hegari (*bmr*6)	4	449.36	11.74	36.88	0.26	449.36	0.24	1882.00	2248.00	388.00	402.50	41.44	4.78	0.52
tube 89	(All x (Ay18 x TAM 2566))24 -1-1-1-1	4	421.08	11.93	37.48	0.28	421.08	0.27	1852.00	3519.00	682.00	361.49	45.85	12.17	1.43
tube 93	Rox Orange (*bmr*6)	4	475.37	17.75	55.77	0.37	475.37	0.52	1550.00	3068.00	466.00	362.45	82.59	18.43	7.74
tube 15	Early Hegari wt deep root	1	507.52	16.07	50.47	0.32	507.52	0.40	2113.00	2658.00	408.00	394.30	98.84	9.90	3.16
tube 94	Kansas Collier (*bmr*6)	4	431.16	14.49	45.51	0.34	431.16	0.38	1810.00	2523.00	387.00	328.55	78.85	18.84	3.74
tube 11	BK7	1	214.33	5.93	18.62	0.28	214.33	0.13	911.00	1446.00	291.00	185.66	21.44	5.47	1.36
tube 65	Kansas Collier (*bmr*12)	3	219.19	10.79	33.89	0.49	219.19	0.42	781.00	1689.00	190.00	150.56	44.02	13.20	5.81
tube 76	Kansas Collier wt	4	199.43	4.68	14.72	0.23	199.43	0.09	684.00	1024.00	238.00	185.84	11.62	1.64	0.25
tube 73	Early Hegari wt deep root	4	548.53	19.15	60.16	0.35	548.53	0.53	1943.00	4139.00	607.00	443.20	78.85	20.63	4.94
tube 40	Rox Orange (*bmr*6)	2	734.87	26.31	82.66	0.36	734.87	0.74	2555.00	5043.00	834.00	577.96	115.64	24.84	12.14
tube 19	Muremb a	1	289.92	8.66	27.22	0.30	289.92	0.20	2159.00	2288.00	374.00	250.35	31.35	4.60	2.38
tube 25	(All x (Ay18 x TAM 2566))25 -1-1-1-1	2	314.66	11.47	36.03	0.36	314.66	0.33	1715.00	2891.00	442.00	245.15	44.70	18.75	5.15
tube 1	Brandes	1	226.61	6.89	21.65	0.30	226.61	0.17	1608.00	1339.00	179.00	195.97	23.07	5.11	1.81
tube 75	Rox Orange (*bmr*12)	4	211.98	6.49	20.40	0.31	211.98	0.16	759.00	1625.00	298.00	176.52	27.42	5.70	1.84
tube 35	BTx 631 wt	2	37.79	1.11	3.50	0.29	37.79	0.03	135.00	195.00	23.00	33.46	3.62	0.40	0.16
tube 12	(All x (Ay18 x TAM 2566))25 -1-1-1-1	1	33.58	1.16	3.64	0.34	33.58	0.03	246.00	193.00	19.00	28.39	3.67	1.33	0.18

tube 50	Muremba	3	312.66	10.84	34.06	0.35	312.66	0.30	1555.00	1941.00	281.00	253.61	42.86	11.22	3.77
tube 33	Kansas Collier (*bmr*6)	2	447.00	13.08	41.10	0.29	447.00	0.30	2317.00	2939.00	559.00	367.16	63.43	12.06	2.88
tube 14	BTx 631 wt	1	5.19	0.11	0.34	0.21	5.19	0.00	34.00	4.00	0.00	5.00	0.19	0.00	0.00
tube 92	(All x (Ay18 x TAM 2566))25-1-1-1-1	4	924.74	28.41	89.25	0.31	924.74	0.69	2865.00	7609.00	1560.00	793.69	89.17	23.79	10.08
tube 69	Mabeyana	3	133.59	3.75	11.79	0.28	133.59	0.08	324.00	750.00	116.00	113.96	17.28	2.27	0.08
tube 34	Rox Orange (*bmr*12)	2	211.03	5.49	17.24	0.26	211.03	0.11	667.00	936.00	214.00	185.08	24.51	1.40	0.03
tube 90	Atlas (*bmr*6)	4	267.05	10.55	33.14	0.40	267.05	0.33	837.00	2701.00	498.00	201.26	45.28	13.05	5.06
tube 26	Mabeyana	2	141.93	6.50	20.41	0.46	141.93	0.23	354.00	1261.00	194.00	100.93	27.14	8.46	3.48
tube 83	Muremba	4	605.95	17.70	55.60	0.29	605.95	0.41	1695.00	2481.00	466.00	518.31	79.56	5.70	1.87
tube 63	Rox Orange wt	3	346.02	9.17	28.80	0.27	346.02	0.19	864.00	1811.00	377.00	304.32	37.40	3.40	0.71
tube 45	Atlas (*bmr*6)	2	296.80	12.97	40.75	0.44	296.80	0.45	915.00	2631.00	395.00	212.84	52.38	17.54	8.99
tube 44	Kansas Collier (*bmr*12)	2	185.37	6.31	19.84	0.34	185.37	0.17	587.00	1332.00	195.00	146.10	30.44	7.13	1.44
tube 71	Early Hegari wt deep root	3	143.92	4.87	15.31	0.34	143.92	0.13	406.00	873.00	152.00	116.55	22.16	4.04	1.02
tube 70	BTx 631(*bmr* 12)	3	79.59	2.58	8.10	0.32	79.59	0.07	458.00	424.00	41.00	63.44	14.61	1.41	0.14
tube 86	Atlas (*bmr*12)	4	266.19	8.35	26.24	0.31	266.19	0.21	850.00	1556.00	232.00	216.18	42.14	6.83	0.64
tube 58	BK7	3	446.72	17.09	53.69	0.38	446.72	0.51	1514.00	3232.00	553.00	337.47	78.42	14.66	7.27
tube 66	Saccaline	3	151.53	4.47	14.04	0.29	151.53	0.10	760.00	926.00	126.00	130.82	16.67	3.06	0.79
tube 8	Atlas(*bmr*6)	1	307.08	8.97	28.18	0.29	307.08	0.21	1068.00	1839.00	440.00	252.95	48.47	5.36	0.27
tube 9	Saccaline	1	462.13	12.37	38.86	0.27	462.13	0.26	1568.00	2025.00	439.00	410.58	44.97	5.57	0.77
tube 18	Early Hegari (*bmr*12)	1	283.57	8.65	27.18	0.31	283.57	0.21	1110.00	1302.00	206.00	234.35	43.55	3.45	1.31
tube 39	Early Hegari wt	2	30.00	0.86	2.71	0.29	30.00	0.02	160.00	64.00	5.00	27.17	2.59	0.23	0.00
tube 38	Kansas Collier wt	2	202.98	5.20	16.33	0.26	202.98	0.11	665.00	869.00	150.00	177.04	22.46	2.91	0.48
tube 46	Early Hegari (*bmr*6)	2	512.54	14.45	45.39	0.28	512.54	0.32	1606.00	2457.00	440.00	455.42	51.83	5.00	0.28
tube 42	Brandes	2	420.13	13.76	43.24	0.33	420.13	0.35	1201.00	2007.00	323.00	331.23	76.95	9.08	1.91
tube 43	(All x (Ay18 x TAM 2566))24-1-1-1-1	2	42.85	0.94	2.96	0.22	42.85	0.02	419.00	53.00	1.00	41.19	1.58	0.04	0.05

tube 78	Early Hegari (*bmr*12)	4	558.87	19.62	61.63	0.35	558.87	0.54	1383.00	3284.00	499.00	453.22	76.50	19.12	7.77
tube 81	Early Hegari (*bmr*12)	4	295.62	9.68	30.41	0.33	295.62	0.25	1067.00	1931.00	275.00	237.62	46.21	7.55	2.12
tube 77	Early Hegari (*bmr*12)	4	6.86	0.31	0.97	0.45	6.86	0.01	73.00	24.00	3.00	4.74	1.36	0.68	0.09
tube 67	Early Hegari (*bmr*6)	3	176.99	5.13	16.10	0.29	176.99	0.12	616.00	1433.00	271.00	153.15	18.97	3.06	1.27
tube 62	M81E	3	72.33	2.37	7.45	0.33	72.33	0.06	259.00	402.00	59.00	59.09	11.70	1.36	0.09
tube 32	BK7	2	540.38	16.38	51.47	0.30	540.38	0.39	1560.00	3764.00	796.00	452.12	73.86	10.18	2.48
tube 72	Early Hegari (*bmr*12)	3	432.81	13.73	43.14	0.32	432.81	0.34	999.00	2202.00	408.00	369.61	53.13	8.05	1.22
tube 89	(All x (Ay18 x TAM 2566))24 -1-1-1-1	4	446.08	13.01	40.86	0.29	446.08	0.30	1015.00	2512.00	506.00	384.00	49.50	10.49	2.06
tube 10	Atlas(*bmr*12)	1	5.46	0.10	0.32	0.19	5.46	0.00	38.00	9.00	2.00	5.38	0.07	0.00	0.00
tube 29	Saccaline	2	287.16	9.42	29.58	0.33	287.16	0.24	1227.00	1960.00	297.00	235.91	38.69	8.06	2.95
tube 2	M81E	1	400.35	11.29	35.47	0.28	400.35	0.25	1567.00	2329.00	399.00	344.14	48.39	6.84	0.74
tube 3	Kansas Collier (*bmr*6)	1	137.69	6.80	21.35	0.49	137.69	0.26	580.00	1671.00	209.00	98.05	18.75	9.84	5.50
tube 5	Kansas Collier wt	1	534.61	15.68	49.24	0.29	534.61	0.36	2139.00	2812.00	558.00	463.25	62.56	6.68	1.55
tube 6	Mabeyana	1	127.18	5.92	18.60	0.47	127.18	0.22	493.00	1090.00	105.00	91.25	23.28	6.91	2.08
tube 23	Early Hegari (*bmr*6)	1	179.61	9.51	29.86	0.53	179.61	0.40	742.00	1803.00	183.00	115.53	35.10	19.30	5.79
tube 96	Brandes	4	362.49	9.62	30.22	0.27	362.49	0.20	1444.00	2266.00	472.00	320.68	34.77	6.36	0.62
tube 7	Kansas Collier (*bmr*12)	1	192.22	5.34	16.76	0.28	192.22	0.12	860.00	1171.00	249.00	166.41	21.04	3.15	1.22
tube 37	Atlas (*bmr*12)	2	19.97	0.53	1.66	0.26	19.97	0.01	163.00	50.00	2.00	18.31	1.50	0.17	0.00
tube 22	Early Hegari wt	1	179.64	4.42	13.88	0.25	179.64	0.09	844.00	1030.00	209.00	165.09	12.56	1.49	0.30
tube 41	Muremba	2	117.82	5.56	17.47	0.47	117.82	0.21	407.00	956.00	100.00	83.27	22.12	9.20	2.09
tube 82	Saccaline	4	397.10	11.76	36.95	0.30	397.10	0.27	1125.00	2134.00	431.00	340.53	41.57	8.47	3.64