

CANDIDATE LIGNOCELLULOSIC BIOREFINERY FEEDSTOCKS FOR  
BIOMASS, SUCCINIC ACID, AND BIOSILICA

A Thesis

by

YIFENG XU

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Chair of Committee,	Russell Jessup
Committee Members,	James Muir
	Jamie Foster

Head of Department,	David D. Baltensperger
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## ABSTRACT

Biofuels produced from non-food lignocellulosic feedstocks have the potential to replace a significant percentage of fossil fuels via high yield potential and suitability for cultivation on marginal lands. Commercialization of dedicated lignocellulosic crops into single biofuels, however, is hampered by conversion technology issues and decreasing oil prices. Integrated biorefinery approaches, where value-added chemicals are produced in conjunction with biofuels, in contrast offer significant potential towards overcoming this economic disadvantage. The objective of this research was to evaluate candidate lignocellulosic feedstocks for potential to produce both primary biofuels and value-added co-products such as succinic acid and biosilica. Feedstock entries included pearl millet napiergrass (PMN), napiergrass, annual sorghum, pearl millet, perennial sorghum, switchgrass, sunn hemp, miscanthus and energy cane. Replicated plots were planted at three locations and characterized for biomass yield, chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF), crude protein (CP), silica, and succinate concentration. The PMN, napiergrass, energy cane and sunn hemp had high biomass yield. They were superior candidates for ethanol production due to high cellulose and hemicellulose yield. They also had high silica and succinate yield with the exception of sunn hemp's low silica yield. Silica yield among feedstock entries ranged from 41 to 3249 kg ha<sup>-1</sup>, and succinate yield ranged from 3 to 556 kg ha<sup>-1</sup>. Therefore, based on high bioethanol and bioproducts yield potential, the

PMN, napiergrass and energy cane are promising biorefinery feedstock candidates for improving biofuel profitability.

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## NOMENCLATURE

PMN	Pearl millet napiergrass hybrid
NDF	Neutral Detergent Fiber
ADL	Acid Detergent Lignin
CP	Crude Protein

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## INTRODUCTION

Interest in finding alternative transportation fuels to fossil fuels has increased due to volatile fossil fuel prices, energy independence and security concerns, and potential long-term environmental impacts of fossil fuels [1]. Bioethanol is by far the most widely used transportation biofuel, which is produced mainly from corn in the USA and sugarcane in Brazil [2]. Global production of bioethanol in 2016 was about 100.3 billion liters, with nearly 56.8 billion liters and 26.5 billion liters produced in the USA and Brazil respectively [3]. With all of the new government programs focusing on renewable energy in America, Asia, and Europe, more than 125 billion liters bioethanol need to be produced by 2020 [4]. Recently, food security risks from first generation biofuel feedstocks such as corn has further spurred interest in alternative renewable resources such as second generation biofuels that are based on non-food lignocellulosic biomass.

Lignocellulosic biomass consists mainly of cellulose, hemicellulose, and lignin in approximately a 4:3:3 ratio that differs across feedstock species. Besides these three components, lignocellulose contain smaller fractions of pectin, protein, pigments and ash [5]. Lignin is often undesirable for bioethanol conversion strategies due to its inhibition to enzymatic saccharification of polysaccharides (cellulose and hemicellulose), and influence on the conversion of simple sugars to desirable fuels and chemicals.

Considering the recalcitrance caused by lignin and the cross-linked structure of lignocellulose, pretreatments of biomass (thermochemical or mechanical) are necessary and much research has been done to test various pretreatment method efficiency and influence on final products (bioethanol and other bio-products). Common pretreatments include dilute acid, hot water, steam explosion and various alkaline processes [6].

Although many pretreatment methods have been evaluated, lignocellulosic biofuel is still far from realizing its industrial production targets. One reason is that current pretreatment technologies still focus on enzymatic hydrolysis and fermentation of cellulose, and the utilization of hemicellulose and lignin, which will contribute to reducing the overall cost, is inhibited by current lignocellulosic component separation technology [5]. Decreases in oil prices have made second generation biofuels less competitive economically to their petroleum counterparts. As a result, commercialization of lignocellulosic biofuels has dramatically declined. Integrated biorefinery approaches, which could produce valuable chemicals along with biofuel, offer significant potential towards overcoming this economic disadvantage. Instead of being used for an energy generation purpose, material products from biorefinery systems, such as building blocks chemicals, organic acids, polymers, resins and so on, can be applied in multiple fields based on their chemical or physical properties [7]. The main purpose of this research was therefore to evaluate candidate lignocellulosic feedstocks for their lignocellulose

composition and potential to produce value-added co-products such as succinic acid and biosilica.

## LITERATURE REVIEW

### Integrated biorefineries

The production of biofuels as a single revenue source remains economically infeasible [8]. The Environmental Protection Agency's (EPA) Renewable Fuel Standard (RFS) program, in consultation with U.S. Department of Agriculture (USDA) and the Department of Energy (DOE), mandated the long-term goal to produce about 136 billion liters of renewable fuel by 2022. Approximately 61 billion liters of this was targeted for production from cellulosic biofuels [9]. However, the EPA significantly reduced the volume requirement for cellulosic biofuel in 2017 from 21 to only 1.2 billion liters [10].

Biorefinery approaches that include diversified output streams by generating both primary biofuels and value-added co-products have significant potential for increased profitability. Conceptual extension of lignocellulosic feedstocks from current single biofuel platforms to integrated biorefineries involve separation and utilization of compositional fractions of biomass into primary biofuels (ethanol from cellulose and hemicellulose, for example) and additional bioproducts (bioplastics, etc.) from the remaining lignin and liquid fractions.

Depolymerization of plant biomass results in primary fractions of cellulose, hemicellulose and lignin. Both cellulose and hemicellulose are polysaccharides, but they

differ in their main components and structures. Cellulose is composed of linear and unbranched chain of  $\beta$ -(1,4)-linked D-glucose, while hemicellulose can be classified to xylan, xyloglucan, glucuronoxylan, arabinoxylan, glucomannan based on its branched chain [5, 6]. Cellulases, as the main enzymes necessary for cellulose hydrolysis, transfers cellulose to hexose. However, a complex enzymatic cocktail is needed for hydrolyzing hemicellulose to pentose [6]. Once the monosaccharide is obtained, downstream fermentation produces ethanol. Unlike cellulose and hemicellulose, lignin is a polyphenolic polymer and often treated as the hindrance for efficient biomass conversion [6]. The majority of lignin is directly combusted for the production of energy during the pulping process, and only small amount has been utilized for conversion into other chemicals. Lignin can, however, be used to produce lignosulfonate. The largest volume of lignosulfonates (50-90%) are utilized as active plasticizing agents in concrete admixture systems as a cost-efficient alternatives to synthetic superplasticizers that are based on fossil fuel sources [11]. The utilization of lignin either directly for biopower or indirectly via upgrading to lignosulfonates, however, has not to date proven sufficient towards making biofuel refineries profitable. Investigation of additional, value-added co-products is therefore warranted.



### Succinic acid

Succinic acid is among the Department of Energy's top value-added chemicals from biomass [12]. Succinic acid is a four-carbon dicarboxylic acid having the potential to be a key building block to make a broad range of products: biodegradable plastics, cosmetics, food ingredients and pharmaceutical products. The petrochemical synthesis of succinic acid includes hydrogenation of 1,4-dicarboxylic unsaturated C<sub>4</sub> acids or anhydrides, oxidation of 1,4-butanediol, and hydrogenation of maleic anhydride to succinic anhydride and then hydration of succinic anhydride to succinic acid [13]. As an alternative process to the petrochemical method, bioproduction of succinic acid from renewable feedstocks by fermentation of glucose using either an engineered form of *Anaerobiospirillum succiniciproducens* and an engineered *Eschericia coli* strain [12]. Recent research has found that high yields and productivities of succinic acid bio-manufacturing can be achieved using *Actinobacillus succinogenes* 130Z in a custom, continuous fermentation step, which makes bio-succinic acid a promising value-added chemical in integrated biorefineries [14]. Increased synthesis and bioaccumulation of such carboxylic acids have also been reported when C<sub>4</sub> perennial grasses are subject to abiotic stress such as drought [15]. These provide opportunity for direct isolation of succinic acid as coproduct from biomass feedstocks without typical lignocellulosic

hydrolysis and upgrading from glucose in competition for use with primary biofuels (bioethanol, etc.).

## Silica

Amorphous silica ( $\text{SiO}_2$ ) is a key material used in diverse semiconductor (photovoltaics, etc.), nanotechnology (fiber optics, nanostructure assembly, microfluidics, etc.), reinforcing agent (resins, plastics, lacquers, coatings, adhesives, paints, printing inks, silicone, etc.), filler (rubber, insulation materials, toothpaste, etc.), and specialty chemical (pharmaceuticals, cosmetics, agrochemicals, etc.) industries. The majority of pure silica is produced today through the smelting of quartz in a high temperature, a relative energy efficient method has been demonstrated can produce pure silica from rice hull ash [16].

Silica within the plant depends on its uptake from the soil, in the form of soluble  $\text{Si}(\text{OH})_4$  or  $\text{Si}(\text{OH})_3\text{O}^-$  [17]. It is ubiquitous across plants, ranging from 0.1 to more than 10% dry weight [17]. Grasses contain the highest silica concentration, which differs among different parts of the same plant (13% in rice straw, 23% in rice hulls and 35% in rice joints)[18]. Silica concentration in perennial grasses like *Panicum maximum* (about 1.07%) and *Pennisetum purpureum* (about 0.85%) are higher than sugarcane bagasse (about 0.44)[19]. High silica concentration in napiergrass (*Pennisetum purpureum*) has

been reported in other research. Four napiergrass varieties showed silica concentration between 0.57 and 4.21%, and higher values were found in the leaves than in the stems [20]. Influences of moisture stress on silica concentration result in silica concentration in napiergrass blades and sheaths up to 5 and 3.4%, respectively [21]. High silica-concentration napiergrass ash has been investigated for its use in many applications including making glass and as an additive for clay ceramics [22, 23]. The median value of Si concentrations in switchgrass samples (1.5%) was 1.4 times higher than that for *M. × giganteus* (1.08%) [24]. The two-step process to isolate lignin and silica from black liquor showed that high silica concentration in the precipitate was achieved at pH 6-7. Below this pH range, silica might get re-dissolved into the solution [25].

## Feedstocks

### *Napiergrass*

Napiergrass is a robust perennial, used primarily as a forage grass in the tropics, that has higher biomass yield potential than most other grasses [26]. As a species native to areas of equatorial Africa where annual precipitation exceeds 1000 mm [27], napiergrass's germplasm varies for cold tolerance, letting this plant has the ability to grow in subtropic areas [26].

Napiergrass is a tetraploid ( $2n=4x=28$ ; A'A' BB) in which the A'A' genome is homeologous to the AA genome of pearl millet (*Pennisetum glaucum* (L.) R. Br.). Napiergrass is protogynous, which facilitates outcrossing. It sets little self-pollinated seed due to self-incompatibility [26].

Napiergrass is typically vegetatively propagated from axillary nodal meristems, whose culms could have more than 20 internodes about 20 to 25 cm long [26]. Greater root mass, root length, shoot mass, and shoot length were obtained from cuttings of the lower and older nodes than the upper and younger nodes, this indicated that cuttings performance was related to cutting maturity [28].

One of the most significant attributes of napiergrass is its high yield potential [27] which also indicates its potential as a bioenergy feedstock. Biomass yields of napiergrass have a wide range dependent on location, cultivar, years since planting, water and soil fertility input levels, among other abiotic factors. Napiergrass biomass production in Gainesville, FL ranged from 24.1 to 27.3 Mg ha<sup>-1</sup> y<sup>-1</sup> in 1986 and 18.5 to 21.1 Mg ha<sup>-1</sup> y<sup>-1</sup> in 1987 [29]. Additional evaluations of 20 napiergrass genotypes at Gainesville, FL, in 1987 reported biomass yields ranged from 8.3 to 24.8 Mg DM ha<sup>-1</sup> y<sup>-1</sup> among the genotypes [30]. A study conducted in Tifton, GA for four consecutive years indicated that napiergrass species Merkeron and N51 produced at least 25 Mg ha<sup>-1</sup> y<sup>-1</sup> biomass for the first 2 y [31]. Trials conducted in Thailand showed that the biomass

yield ranged from 27.1 to 58.4 t ha<sup>-1</sup> y<sup>-1</sup> among eight napiergrass accessions [32]. This study also investigated the influence of dry season and rainy season on cellulose and lignin content of those cultivars. The high biomass accumulation rates consistently reported for napiergrass support its use as a biofuel feedstock.

*‘PMN’ (Pearl Millet-Napiergrass)*

Napiergrass (2n=4x=28) can be crossed with pearl millet (2n=2x=14) to produce interspecific triploid hybrids (2n=3x=21). These triploid hybrid can combine the forage quality of pearl millet and biomass yield potential of napiergrass [26]. Pearl millet-napiergrass hybrid (“PMN”; *Pennisetum glaucum* [L.] R. Br. × *P. purpureum* Schumach.) is also a candidate biofuel feedstock due to its sterile F<sub>1</sub> hybrids that can be planted via seed and produce high biomass yields beginning in the establishment year. Pearl millet-napiergrass can have both high yields of perennial grasses like energycane (*Saccharum* spp.) and large seed of annual grasses such as sorghum (*Sorghum bicolor* [L.] Moench) [33]. Seed production of pearl millet-napiergrass is equivalent to that of commercial forage sorghum [34]. The large yield and size of PMN seed make its establishment more cost-effective than the vegetative reproduction of perennial species such as energycane and giant Miscanthus (*Miscanthus x giganteus*) [35]. Establishment year yields of PMN reach 37 Mg DM ha<sup>-1</sup> y<sup>-1</sup> in subtropical climates [36].

### *Pearl millet*

Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is an annual sexual diploid ( $2n=2x=14$ ). Pearl millet originated in northern Africa across from western Sudan to Senegal [37]. This annual grass is mainly used as grain crop in India, Pakistan, and Africa; however, its main usage in the USA, Australia, and parts of South America is as a forage [38]. There is growing interest in planting this grass as a grain crop to feed livestock in the USA [38]. Although grain production of pearl millet hybrid is not competitive to sorghum and maize hybrids, as a forage crop its yield can exceed sorghum and maize [39]. One study comparing forage yield among sorghum, millet and corn cultivars found higher biomass yield in pearl millet ( $12,285 \text{ kg ha}^{-1}$ ) than all three sorghum cultivars (Jumbo, Speed feed, Sugar graze) [40]. Pearl millet is adapted to poor, droughty and infertile soil conditions which are too harsh for other grain crops. Once high fertility and moisture is available, pearl millet can respond quickly [38].

### *Switchgrass*

Switchgrass (*Panicum virgatum* L.) is a perennial grass indigenous across North America. Switchgrass can be utilized as a forage crop, either grazed or harvested for making silage and hay [41]. In the USA, significant recent research on herbaceous energy crop production systems has focused on switchgrass [42] due to its wide

adaptation, genetic diversity, biomass yield potential, and suitability for marginal land. Biomass yields of switchgrass in relatively poor environments emphasize that its potential range of adaptation to be quite large and diverse [43]. The suitability of switchgrass for marginal land makes it a good biofuel candidate feedstock across the approximately 19.4 million hectares of marginal land not capable of growing conventional crop [44].

Among switchgrass cultivars, ‘Alamo’ has been found to have higher biomass yield and broader adaptability than others in Virginia, Alabama and Texas [45]. For sustainable biomass production, N application ( $168 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ), but not P application and row spacing, is necessary for switchgrass to achieve an average biomass production of  $14.5$  and  $10.7 \text{ Mg DM ha}^{-1} \text{ y}^{-1}$  at Stephenville and Beeville, TX, respectively [46]. Other research also indicates that N application influences biomass yield of switchgrass [47, 48].

### *Miscanthus*

Giant miscanthus is a perennial  $C_4$  grass native to Asia. It is a triploid crossing of *M. sinensis* (a diploid species) and *M. sacchariflorus* (a tetraploid species) [49]. This triploid warm-season  $C_4$  grass is unable to produce seeds and must be propagated by rhizomes. Significant research on giant miscanthus as a bioenergy grass has been done

in Europe, and its biomass yield compares to U.S. DOE's model energy grass—switchgrass [50]. Giant miscanthus's average biomass yields can reach 28.7 t DM ha<sup>-1</sup> y<sup>-1</sup> when cultivated in the temperate climate of central Italy [51]. High average biomass yields, excluding the two establishment years, ranged from 22.0 to 35.4 Mg ha<sup>-1</sup> y<sup>-1</sup> in Illinois [52]. However, this high biomass yield cannot be reached in Texas [53]. A quantitative review comparing the yield potential between giant micanthus and switchgrass indicated that first will yield 12 Mg more biomass ha<sup>-1</sup> but its yield is more affected by water availability than the latter across a wide range of growing conditions [48]. Side-by-side experiments conducted in the USA comparing biomass yield of switchgrass and giant miscanthus can be found in some recent research [54, 55].

### *Energy cane*

Sugarcane (*Saccharum* L. spp.) is a perennial grass cultured mainly for sucrose production [56]. Energy cane has more fiber but less sucrose than sugarcane [57]. A review on energy cane bioenergy industry potential described in detail the transferring interest from sugarcane to energy cane [57]. As a perennial bioenergy crop derived from sugarcane, energy cane has higher fiber concentration, higher biomass yields, and better cold tolerance than sugarcane [58]. Improved cold tolerance allows energy cane to extend its growing regions further north than that of sugarcane in the USA (Florida,



Louisiana, and Texas) [59]. More importantly, its ability to grow on marginal land will give it more potential as a lignocellulosic biomass feedstock [60]. Biomass yields of energy cane have been comparable to other lignocellulose feedstock in different research [36, 61]. Biomass yield and composition are influenced by harvest frequency and timing [58, 62]. Legendre and Burner found that first generation hybrids ( $F_1$ ) were best suited for energy cane, backcrosses reduced biomass yield components, and the greater the number of backcrosses the higher that dilution [56].

### *Sorghum*

Sorghum is an important cultivated crop used mainly for grain and forage production, and it is now being treated as a bioenergy crop [63]. Its diverse utilization, including bioenergy potential of grain sorghum, sweet sorghum and high biomass cellulosic sorghum (including bagasse, residue, high-biomass sorghums and photoperiod sensitive sorghums), is significant [63]. Sweet sorghum (*Sorghum bicolor L.*) is an excellent annual bioenergy feedstock due to its complementary harvest window to sugarcane which makes near year-round operation of conversion facilities feasible and its potential to produce significant amounts of free sugars for fermentation [64].

Among many traits of forage sorghum that may impact forage quality, one trait is controlled by brown midrib (BMR) mutants, which influences forage quality [65].

Chemically induced BMR mutants in sorghum were first induced in 1978 and can reduce lignin concentration as high as 51% in stems and 25% in leaves [66]. With the reduced lignin concentration, BMR sorghum cultivars have greater forage digestibility [65]. More importantly from a bioenergy perspective, this attribute could improve overall cellulosic ethanol conversion efficiencies when recalcitrance caused by lignin is taken into account. Brown midrib mutant effect on lignin concentration reduction, glucose yields and conversion of cellulose to ethanol has been investigated using wild type, BMR-6, BMR-12, and BMR-6 BMR-12 double mutant species [67]. Biomass and estimated ethanol yields among sweet sorghum, BMR sorghum cultivars, and several perennial grasses indicated that the highest biomass yield and ethanol production can be achieved by sweet sorghum [68].

### *Sunn hemp*

Sunn hemp (*Crotalaria juncea* L.) is a legume native to India used as a soil-improving crop, a green manure, and as livestock feed [69]. Due to its fast growing and the ability to fix N and to control weeds and nematodes, 'Tropic Sun' is used as a green manure for rotation with many plants [70]. High biomass yield to prevent soil erosion and significant N releasing to following crops make sunn hemp an alternative to winter legume in warm temperate regions [71]. Its potential to replace winter legumes as a

cover crop has focused on dry biomass, N accumulation, and decomposition of residue [71-73]. Although there is wide interest in sunn hemp as a cover crop, limited research has been done to evaluate sunn hemp's bioenergy potential. Sunn hemp's potential as a biofuel feedstock vis-à-vis forage sorghum was tested by comparing the end-product after dilute acid pretreatment and enzymatic hydrolysis [74]. Due to its ability to accumulate large amounts of biomass in a short time frame, one study conducted to evaluate it as a bioenergy feedstock found sunn hemp produced 10.7 Mg DM ha<sup>-1</sup>, after 12 weeks growth, which equals to 204 GJ ha<sup>-1</sup> energy yield [75].

## OBJECTIVES

Characterize biomass yields and chemical composition, including hemicellulose, cellulose, acid detergent lignin, neutral detergent fiber, crude protein, succinic acid and biosilica concentration in diverse feedstocks.

Characterize chemical composition including hemicellulose, cellulose, acid detergent lignin, neutral detergent fiber, crude protein and succinic acid concentration under drought stressed and non-drought stressed conditions in diverse feedstocks.

## MATERIALS AND METHODS

### Plant entries

- 1) Pearl Millet-Napiergrass (PMN) hybrid PMN10TX13,
- 2) Napiergrass cultivar Merkeron,
- 3) Napiergrass accession PEPU 09FL03,
- 4) Napiergrass accession PEPU 09FL01,
- 5) SDH2942 BMR sorghum,
- 6) Annual sorghum cultivar SX-17,
- 7) Exceed BMR Pearl millet,
- 8) Perennial sorghum hybrid PSH 09TX15,
- 9) Switchgrass cultivar Alamo,
- 10) Sunn hemp cultivar 'Tropical Isle',
- 11) Giant miscanthus (Mxg),
- 12) Energy cane (unknown accession).

## Field evaluation

### *Propagation of planting materials*

The stalks of two napiergrass accessions (PEPU 09FL01, PEPU 09FL03) were harvested in October 2015 from College Station, TX, fields. Nodes cut from the stalks were planted in barrels in greenhouse for propagating into trays in spring 2016. The rhizome of PSH 09TX15 perennial sorghum was collected in the same time and increased in a 95 l barrel in a greenhouse. In Spring 2016, those two napiergrass cultivars, Merkeron, one energycane species, the perennial sorghum and giant miscanthus were transplanted into propagation trays. Then they were acclimatized outside for 3 wk in April 2016.

### *Field planting*

In May 2016, replicated plots (n=3) were planted in a completely randomized design in College Station, Beeville, and Stephenville, TX. The College Station location (30°32'N, 96°26'W; elevation 81m) was on a Weswood silty clay loam (pH 8.0). The Beeville location (28°27'N, 97°42'W; elevation 70 m) was on a Parrita sandy clay loam (pH 7.2). The Stephenville location (34°17'N, 96°12'W; elevation 370 m) was on a Windthorst fine sandy loam (pH 6.8). Each cultivar was planted in three plots (3 x 3 m)

with four, 3-m rows. Entries 1,2,3,4,8,9,11 and 12 were planted vegetatively with seven plants in each row except that Alamo was previously planted with six plants in each row. Entries 5, 6, 7 and 10 were planted by seeds using a Jang JP-1 roller-type seeder. Weed control was conducted by hand and mechanical cultivation. Total of 43.5" of water were applied (31.5" of rainfall and 12" of irrigation) during the growing season at College Station. Total of 25.8" of water were applied (7.8" of rainfall and 18" of irrigation) at Stephenville. And total of 23.7" of water were applied (17.7" of rainfall and 6" of irrigation) at Beeville. For nitrogen fertilization, a single application of 80 lbs N per acre (urea) was made 3 wk post planting.

#### *Harvesting and estimation of biomass yield*

Field harvests were done in November 2016. One of the two center rows of each plot was harvested, the whole weight of the 3-m row harvested was measured. A subsample was obtained from each plot and air dried to determine biomass yield. Leaf samples were collected and packed in dry ice before being frozen for succinic acid content assay. The leaf samples were kept frozen (-20 °C) until analyzed. The air-dried subsamples were first ground through a hammer mill and then ground through a 1-mm sieve in a Wiley mill (Thomas Scientific, Philadelphia, PA) for lignocellulosic composition and bio-silica analysis.

#### *Chemical composition (NDF, ADF, ADL, CP)*

Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) was determined successively according to methodology described by Van Soest and Robertson (1980), modified by using an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY). Neutral detergent fiber is the residue remaining after digesting in a detergent solution, which are mainly hemicellulose, cellulose, and lignin according to Van Soest and Robertson (1980). Acid detergent fiber is the residue, remaining after digesting with  $\text{H}_2\text{SO}_4$  and CTAB, which are predominantly cellulose and lignin [76]. Lastly, acid detergent lignin is the residue remaining after digesting with 72% sulfuric acid [77]. This analysis was performed sequentially on ADF residue. Nitrogen (N) was determined using an elemental analyzer (Vario Macro, Elementar, Germany) and crude protein (CP) was calculated as 6.25 N.

#### *Succinic acid (succinate) analysis*

Leaf samples collected from field trials in September and November 2016 were used for succinic acid content assays. Measurement of succinate or succinic acid was conducted using the Succinate (Succinic Acid) Colorimetric Assay kit (Biovision, Milpitas, California, USA), according to manufacturers' instructions. At first, succinyl-CoA synthetase transfers succinate to form an intermediate, which then goes through a



series of reactions and reduces a colorless probe to a colored product. Then the absorbance can be detected around 450 nm. This kit was used to detect the intracellular concentration of succinate in an experiment [78].

### *Silica analysis*

Silica was tested according to Reidinger et al. (2012), using a portable X-ray fluorescence spectrometer (DELTA Premium, OLYMPUS, Tokyo, Japan). This method requires relatively small amounts of plant material and is an accurate and quick technique for detecting silica content in plants [79]. In order to test the accuracy of this experiment, Si calibration standards were made by first mixing synthetic methyl cellulose and silica powder and then homogenizing them to produce standard with 0%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% silica concentration. Pellets of both Si calibration standard and dried and ground plant samples were made by pressing 1.0 g of each substance at 12 Mg using a manual hydraulic press. Then pellets were analyzed for silica content by using X-ray fluorescence spectrometer.

### Greenhouse evaluation

To evaluate the drought induction of succinic acid accumulation, all 12 cultivars were planted in the greenhouse in May 2016. Replicated plots (n=3) were planted in

completely randomized design in two sides of the greenhouse. A potting media mix (Sunshine Redi-Earth Plug & Seed Potting Mix) was used. Plants were maintained in the greenhouse for 3 mo before drought treatments were imposed to allow acclimation of plants to the greenhouse conditions. Treatments included a well-watered control and deficit irrigation regime. Soil volumetric water content (SWC) were measured before irrigation using a soil moisture meter (FieldScout TDR 100; Spectrum Technologies). Well-watered plants were irrigated to maintain soil water content at field capacity. In the drought stress treatment, plants were not watered until the volumetric soil moisture content falls below 15%.

Greenhouse plants were harvest in late November 2016 for DM calculation. Leaf samples were collected. The air-dried subsamples were first ground through a hammer mill, dried in a forced-air oven at 55°C until weight loss ceases and then ground through a 1-mm sieve in a Wiley mill (Thomas Scientific, Philadelphia, PA). Chemical composition (NDF, ADF, ADL, CP and succinic acid) was analyzed as previously described.

#### Data analysis and statistics

The experimental design for both the field trial and the greenhouse trial was completely randomized design with three replications. Individual pots in the greenhouse

and plots in the field were experimental units. The field statistical model consisted of location, harvest and plant entry in a three-factorial arrangement looking at three-way interactions and, if those were not significant, at simple effects. The greenhouse statistical model consisted of irrigation level and plant entry in a two-factorial arrangement looking at two-way interactions and, if those were not significant, at simple effects. Dependent variables included biomass yield, hemicellulose, cellulose, NDF, ADL, CP, succinic acid and silica concentration. Data collected was submitted to analysis of variance and, where appropriate, multiple means separated using All Pair, Turkey HSD with JMP software (JMP Pro12, Statistical Analysis System, USA). Differences were considered significant at  $P \leq 0.05$  but values above this were reported for each analysis.

## RESULTS AND DISCUSSION

### Field evaluation

#### *Statistical analysis*

Differences were identified at all locations for every trait evaluated except NDF (Table 1). Time of harvest and feedstock entry effects also varied. Interactions between location and harvest time occurred for cellulose, ADL, CP and silica. Location by feedstock entries interacted across all traits. Harvest time by feedstock entries interacted for cellulose, ADL, NDF and silica. A three-way interaction between location, harvest time and feedstock entries was also identified for silica and succinate content.

Table 1. Analysis of variance of biomass yield, hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF), crude protein (CP), silica and succinate concentration for field experiment.

	Biomass	Hemicellulose	Cellulose	ADL	NDF	CP	Silica	Succinate
loc <sup>Y</sup>	**Z	***	*	***	ns	***	***	***
time <sup>X</sup>	-	***	***	***	***	***	***	ns
trt <sup>W</sup>	***	***	***	***	***	***	***	***
loc*time	-	ns	**	**	ns	**	**	***
loc*trt	***	**	***	**	***	***	*	***
time*trt	-	ns	***	*	***	ns	***	***
loc*time*trt	-	ns	ns	ns	ns	ns	**	***

<sup>Z</sup> NS (nonsignificant) or significant at  $P \leq 0.05$  (\*), 0.01 (\*\*), or 0.001 (\*\*\*)

<sup>Y</sup> location: Stephenville, Beeville, and College Station TX

<sup>X</sup> Harvest time: September and November in 2016

<sup>W</sup> Plant entry

*Early Fall (September): Chemical composition*

Giant miscanthus had the highest hemicellulose concentration at all locations (Table 2, 3, 4). Pearl millet was equivalent at Stephenville and Beeville, and switchgrass was equivalent at Beeville. Sunn hemp had the lowest hemicellulose concentration at Stephenville and College Station; however, due to small ruminant predation, it was lost at Beeville. Cellulose concentration was uniform across most feedstock entries at all locations. Pearl millet, in contrast was lower at all locations. Sunn hemp had the highest lignin concentration in Stephenville and College Station, while SX-17 sorghum had the highest value in Beeville. The BMR pearl millet entry had the lowest lignin concentration at all three locations. The perennial sorghum entry was higher in NDF at all locations; however, numerous entries were similar. Sunn hemp possessed the highest CP concentration at Stephenville and College Station. Entries were not different at Beeville.

Annual BMR sorghum had the highest silica concentration at all three locations (Table 2, 3, 4). Sunn hemp had the lowest silica concentration at the two locations where it survived. Overall silica concentration across all entries was lower at Stephenville compared to College Station and Beeville.

The succinate concentration of the BMR pearl millet was highest among feedstock entries at Stephenville and College Station (Table 2, 3). Two napiergrass

entries (PEPU 09FL03, PEPU 09FL01) were equivalent to the BMR pearl millet at Stephenville, but another napiergrass (Merkeron) was equivalent to it at College Station.

Table 2. Stephenville September sample of chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF) (g kg<sup>-1</sup> biomass), crude protein (CP) (%), silica (%) and succinate (g kg<sup>-1</sup> leaf).

Entry	Means						
	Hemicellulose	Cellulose	ADL	NDF	CP	Si	Succinate
	g kg <sup>-1</sup>				%		g kg <sup>-1</sup>
PMN 10TX13 <sup>Y</sup>	265 de <sup>Z</sup>	366 ab	40.4 bcd	672 abc	6.31 bcd	3.63 abc	0.3 c
Merkeron <sup>X</sup>	270 cde	353 abc	32.6 bcd	656 bc	7.06 bc	3.69 abc	3.8 bc
PEPU 09FL03 <sup>W</sup>	269 cde	355 abc	47.1 bcd	671 abc	6 bcd	4.16 ab	7.4 a
PEPU 09FL01 <sup>W</sup>	260 e	395 a	56.4 bc	711 ab	4.81 cd	3.07 bc	8.4 a
BMR Sorghum <sup>V</sup>	260 e	339 bc	23.8 cd	623 cd	6.44 bcd	4.86 a	6.1 ab
SX-17 <sup>U</sup>	272 bcde	362 abc	50.3 bcd	685 abc	5.31 cd	4.41 ab	1.6 c
BMR pearl millet <sup>T</sup>	307 a	321 c	21.2 d	649 bc	7.19 bc	2.48 c	8.8 a
PSH 09TX15 <sup>S</sup>	291 abcd	389 a	40.8 bcd	721 a	4.44 d	3.6 abc	0.5 c
Alamo switchgrass	301 ab	346 bc	62.5 ab	709 ab	5.13 cd	2.98 bc	6.3 ab
Tropical isle Sunn Hemp	107 f	365 ab	90.0 a	561 d	13.44 a	0.51 d	6 ab
Giant miscanthus (Mxg)	303 a	353 abc	35.6 bcd	691 ab	8.19 b	4.32 ab	0.3 c
Energy cane <sup>R</sup>	298 abc	376 ab	35.8 bcd	710 ab	6.31 bcd	3.93 abc	6.1 ab

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Table 3. College Station September sample of chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF) (g kg<sup>-1</sup> biomass), crude protein (CP) (%), silica (%) and succinate (g kg<sup>-1</sup> leaf).

Entry	Means						
	Hemicellulose	Cellulose	ADL	NDF	CP	Si	Succinate
	g kg <sup>-1</sup>				%		g kg <sup>-1</sup>
PMN 10TX13 <sup>Y</sup>	225 d <sup>Z</sup>	409 ab	79.7 ab	714 a	3.06 e	4.34 bc	4.3 abc
Merkeron <sup>X</sup>	265 bcd	319 de	50.3 bc	634 b	5.51 bcd	5.33 ab	5.2 a
PEPU 09FL03 <sup>W</sup>	242 cd	356 bcd	64.3 bc	663 ab	4.37 de	5.34 ab	4.7 ab
PEPU 09FL01 <sup>W</sup>	249 bcd	350 cde	65.1 bc	664 ab	4.62 cde	4.9 abc	4.3 abc
BMR Sorghum <sup>V</sup>	238 d	379 bc	47.3 bc	664 ab	3.69 e	6.68 a	1.9 c
SX-17 <sup>U</sup>	258 bcd	389 abc	64.5 bc	711 a	3.13 e	4.49 bc	3.1 abc
BMR pearl millet <sup>T</sup>	292 ab	297 e	38.9 c	627 b	6.24 abc	3.28 c	5.5 a
PSH 09TX15 <sup>S</sup>	267 bcd	384 abc	57.1 bc	708 a	4.16 de	5.79 ab	2.8 abc
Alamo switchgrass	282 abc	347 cde	67.6 bc	696 a	3.98 de	3.19 c	3.4 abc
Tropical isle Sunn Hemp	124 e	442 a	111 a	677 ab	7.8 a	0.53 d	1.7 bc
Giant miscanthus (Mxg)	320 a	338 cde	39.4 c	697 a	6.8 ab	4.61 bc	3.2 abc
Energy cane <sup>R</sup>	266 bcd	361 bcd	46.1c	673 ab	3.86 de	4.21 bc	3.9 abc

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Table 4. Beeville September sample of chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF) ( $\text{g kg}^{-1}$  biomass), crude protein (CP) (%), silica (%) and succinate ( $\text{g kg}^{-1}$  leaf).

Entry	Means						
	Hemicellulose	Cellulose	ADL	NDF	CP	Si	Succinate
	$\text{g kg}^{-1}$				%		$\text{g kg}^{-1}$
PMN 10TX13 <sup>Y</sup>	243 d <sup>Z</sup>	372 ab	53.2 ab	668 bc	6.82 a	6.06 ab	11.3 a
Merkeron <sup>X</sup>	260 bcd	345 ab	55.2 ab	660 bc	6.53 a	5.18 b	10.9 a
PEPU 09FL03 <sup>W</sup>	261 bcd	358 ab	54.4 ab	673 bc	6.00 a	5.44 ab	12.0 a
PEPU 09FL01 <sup>W</sup>	264 bcd	379 ab	60.0 ab	703 ab	6.22 a	4.7 b	11.1 a
BMR Sorghum <sup>V</sup>	243 d	359 ab	33.8 ab	636 c	5.38 a	7.63 a	11.8 a
SX-17 <sup>U</sup>	258 cd	377 ab	64.7 a	700 ab	3.99 a	4.6 b	10.1 a
BMR pearl millet <sup>T</sup>	298 a	331 b	31.5 b	661 bc	7.31 a	4.38 b	10.7 a
PSH 09TX15 <sup>S</sup>	280 abc	387 a	59.2 ab	726 a	5.74 a	4.74 b	12.2 a
Alamo switchgrass	299 a	339 ab	54.7 ab	693 ab	5.8 a	4.16 b	12.0 a
Tropical isle Sunn Hemp							9.3 ab
Giant miscanthus (Mxg)	301 a	340 ab	40.6 ab	682 abc	7.34 a	5.56 ab	2.6 b
Energy cane <sup>R</sup>	282 ab	362 ab	43.1 ab	687 abc	5.95 a	4.95b	8.1 ab

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.



*Late Fall (November): Biomass yield*

Biomass yield varied across location and feedstock entries (Table 1). At Stephenville, energy cane had the highest yield but was closely followed by first year PMN, all napiergrass entries, both annual sorghum entries, and sunn hemp (Table 5). Giant miscanthus and pearl millet had the lowest yield. Despite being in its third season, switchgrass was low to intermediate. At College Station, third season PMN had the highest yield. Two napiergrass entries (Merkeron, PEPU 09FL03), energy cane, and sunn hemp had intermediate yields at this location (Table 6). All the remaining entries including third season switchgrass had low yield. Third season PMN also had the highest yield at Beeville, while there were no difference for the remaining entries (Table 7).

*Late Fall (November): Chemical composition*

Giant miscanthus had the highest hemicellulose concentration in College Station and Beeville (Table 6, 7), while in Stephenville pearl millet possessed the highest hemicellulose concentration (Table 5). At all locations, sunn hemp had the lowest hemicellulose concentration. Sunn hemp had the highest cellulose concentration in Stephenville and College Station. Across most remaining entries, cellulose concentration were highly uniform at all locations. Sunn hemp had the highest ADL at all locations. One napiergrass entry (PEPU 09FL01) was equivalent in Beeville. The BMR sorghum had the lowest ADL in College Station and Beeville, while BMR pearl millet had the lowest ADL in Stephenville. The perennial sorghum entry had the highest NDF at Stephenville and Beeville, while the NDF of the non-BMR sorghum was the highest in

College Station. The NDF of pearl millet was the lowest in Stephenville and College Station, however, in Beeville, the BMR sorghum had the lowest NDF. Crude protein of Sunn hemp was higher than other feedstock entries across all locations.

Across all locations, the BMR sorghum had the highest silica concentration, while sunn hemp was the lowest in silica concentration. However, the silica yield ( $\text{kg ha}^{-1}$ ) of the BMR sorghum tended to range from intermediate to low at all locations (Table 8a) and the silica yield of the perennial sorghum and switchgrass was equivalent to the BMR sorghum. The silica yield of the third year PMN was the highest in College Station and Beeville. And in Stephenville, even in its first year, the PMN possessed the highest silica yield, with similar silica yield from one napiergrass accession (PEPU 09FL03) and the energy cane.

There were no differences for succinate concentration among feedstock entries at all locations (Table 5, 6, 7). However, the third year PMN had the highest succinate yield ( $\text{kg ha}^{-1}$ ) at College Station and Beeville (Table 8b). At Stephenville, PEPU 09FL03 had the highest succinate yield, while the first year PMN and the energy cane was equivalent.

Table 5. Stephenville November sample traits of biomass yield (kg ha<sup>-1</sup>) and chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF) (g kg<sup>-1</sup> biomass), crude protein (CP) (%), silica (%) and succinate (g kg<sup>-1</sup> leaf).

Entry	Means							
	DM	Hemicellulose	Cellulose	ADL	NDF	CP	Si	Succinate
	kg ha <sup>-1</sup>	g kg <sup>-1</sup>				%		g kg <sup>-1</sup>
PMN 10TX13 <sup>Y</sup>	30975 ab <sup>Z</sup>	247 c	404 bc	64.2 abc	716 abcd	4.06 bcd	4.11 ab	7.3 a
Merkeron <sup>X</sup>	23589 abc	245 c	398 bc	68.9 abc	712 abcd	3.44 bcd	2.68 c	8.3 a
PEPU 09FL03 <sup>W</sup>	32469 ab	261 bc	378 bcde	71.5 abc	711 bcd	3.06 cd	3.58 bc	8.2 a
PEPU 09FL01 <sup>W</sup>	17955 abc	245 c	402 bc	87.4 ab	734 abc	2.69 d	3.48 bc	8.5 a
BMR Sorghum <sup>V</sup>	8746 abc	278 abc	314 e	70.3 abc	662 cd	4.44 abcd	5.36 a	5.6 a
SX-17 <sup>U</sup>	16912 abc	260 abc	387 bcd	67.4 abc	715 abcd	2.63 d	3.54 bc	6.9 a
BMR pearl millet <sup>T</sup>	7804 c	306 a	333 de	29.4 c	668 d	5 ab	2.73 c	6.4 a
PSH 09TX15 <sup>S</sup>	10872 bc	289 ab	413 b	65.1 abc	767 a	2.5 d	4.91 a	8.0 a
Alamo switchgrass	11717 bc	297 ab	369 bcde	64.7 abc	730 abc	3.31 bcd	2.98 c	7.3 a
Tropical Isle Sunn Hemp	31774 ab	144 d	507 a	98.5 a	749 ab	6.56 a	0.4 d	5.7 a
Giant miscanthus (Mxg)	4453 c	285 abc	356 cde	64.9 abc	705 bcd	4.75 abc	4.42 ab	5.6 a
Energy cane <sup>R</sup>	33044 a	260 bc	371 bcde	50.1 bc	681 cd	3.38 bcd	3.04 c	7.2 a

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Table 6. College Station November sample traits of biomass yield (kg ha<sup>-1</sup>) and chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF) (g kg<sup>-1</sup> biomass), crude protein (CP) (%), silica (%) and succinate (g kg<sup>-1</sup> leaf).

Entry	Means							
	DM	Hemicellulose	Cellulose	ADL	NDF	CP	Si	Succinate
	kg ha <sup>-1</sup>	g kg <sup>-1</sup>				%		g kg <sup>-1</sup>
PMN 10TX13 <sup>Y</sup>	71318 a <sup>Z</sup>	216 b	401 bcd	97.2 ab	714 ab	2.25 d	4.2 bcd	5.1 a
Merkeron <sup>X</sup>	16442 bc	241 ab	417 bc	67.5 cdef	726 ab	3.31 bcd	3.85 bcd	5.4 a
PEPU 09FL03 <sup>W</sup>	9461 bc	235 ab	374 cd	90.7 abc	700 ab	2.6 bcd	3.62 bcd	5.9 a
PEPU 09FL01 <sup>W</sup>	6000 c	258 ab	375 cd	69.6 cdef	702 ab	3.15 bcd	4.22 bcd	5.6 a
BMR Sorghum <sup>V</sup>	3338 c	255 ab	416 bc	35.5 g	706 ab	3.25 bcd	6.74 a	4.5 a
SX-17 <sup>U</sup>	4316 c	241 ab	438 b	70.4 cdef	749 a	2.41 cd	3.98 bcd	3.8 a
BMR pearl millet <sup>T</sup>	1567 c	276 a	358 d	46.4 efg	680 b	4.51 abc	3.53 cd	4.0 a
PSH 09TX15 <sup>S</sup>	3961 c	243 ab	428 b	71.5 cde	742 ab	3.07 bcd	5.21 ab	3.5 a
Alamo switchgrass	6015 c	289 a	372 cd	77.6 bcd	738 ab	2.64 bcd	2.93 d	4.9 a
Tropical Isle Sunn Hemp	25820 b	120 c	505 a	105 a	730 ab	5.48 a	0.49 e	2.5 a
Giant miscanthus (Mxg)	2328 c	289 a	361 d	56.8 defg	707 ab	4.69 ab	4.61 bc	3.6 a
Energy cane <sup>R</sup>	13704 bc	266 ab	376 cd	45 fg	687 ab	3.21 bcd	3.77 bcd	3.7 a

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Table 7. Beeville November sample traits of biomass yield (kg ha<sup>-1</sup>) and chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF) (g kg<sup>-1</sup> biomass), crude protein (CP) (%), silica (%) and succinate (g kg<sup>-1</sup> leaf).

Entry	Means							
	DM	Hemicellulose	Cellulose	ADL	NDF	CP	Si	Succinate
	kg ha <sup>-1</sup>	g kg <sup>-1</sup>				%		g kg <sup>-1</sup>
PMN 10TX13 <sup>Y</sup>	69519 a <sup>Z</sup>	232 d	422 a	74.9 abc	728 ab	5.86 b	3.11 de	8.0 a
Merkeron <sup>X</sup>	18955 b	255 abcd	414 ab	65.2 bcd	734 ab	3.82 bcd	4.38 abcd	7.6 a
PEPU 09FL03 <sup>W</sup>	15635 b	250 abcd	388 abcd	90.9 ab	729 ab	3.35 cd	4.15 bcd	9.3 a
PEPU 09FL01 <sup>W</sup>	12803 b	244 bcd	414 ab	99.6 a	758 a	3.1 cd	3.86 cd	9.0 a
BMR Sorghum <sup>V</sup>	6678 b	240 cd	367 bcd	40.5 d	648 c	3.92 bcd	6.05 a	6.7 a
SX-17 <sup>U</sup>	13260 b	239 cd	400 abc	62.7 bcd	701 b	3.32 cd	3.65 de	7.7 a
BMR pearl millet <sup>T</sup>	919 b	278 abc	380 abcd	46.5 cd	705 abc	4.94 bcd	4.4 abcd	7.3 a
PSH 09TX15 <sup>S</sup>	9961 b	265 abcd	433 a	61.8 bcd	760 a	2.6 d	5.58 abc	9.0 a
Alamo switchgrass	6137 b	282 ab	359 cd	62.5 bcd	704 b	3.53 bcd	2.97 de	8.9 a
Tropical Isle Sunn Hemp		115 e	439 ab	111 a	665 bc	10.3 a	1.03 e	8.3 a
Giant miscanthus (Mxg)	1624 b	289 a	348 d	52.9 cd	690 bc	5.34 bc	5.83 ab	6.3 a
Energy cane <sup>R</sup>	14239 b	266 abcd	383 abcd	57.2 cd	706 b	4.26 bcd	4.3 abcd	6.1 a

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Table 8. Bioproduct yield in November 2016 at Stephenville, College Station and Beeville

a. Silica yield (kg ha<sup>-1</sup>)

Entry	Means		
	Stephenville	College Station	Beeville
PMN 10TX13 <sup>Y</sup>	1281 a <sup>Z</sup>	3249 a	2211 a
Merkeron <sup>X</sup>	631 abc	645 b	825 b
PEPU 09FL03 <sup>W</sup>	1264 ab	311 b	639 b
PEPU 09FL01 <sup>W</sup>	627 abc	247 b	494 b
BMR sorghum <sup>V</sup>	468 abc	238 b	412 b
SX-17 <sup>U</sup>	577 abc	175 b	473 b
BMR pearl millet <sup>T</sup>	212 c	53 b	41 b
PSH 09TX15 <sup>S</sup>	542 abc	200 b	559 b
Alamo switchgrass	350 bc	178 b	181 b
Tropical Isle Sunn Hemp	144 bc	90 b	-
Giant miscanthus (Mxg)	198 c	106 b	100 b
Energy cane <sup>R</sup>	1008 abc	518 b	613 b

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Table 8. Continued

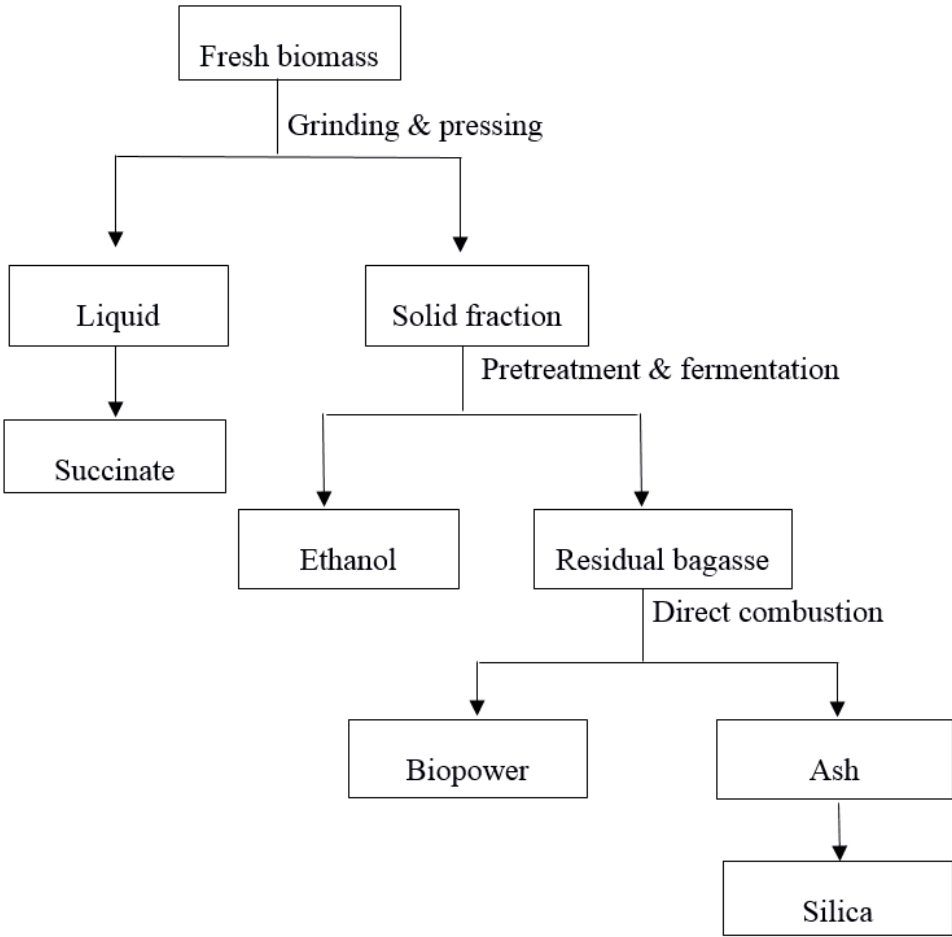
b. Succinate yield (kg ha<sup>-1</sup>)

Entry	Means		
	Stephenville	College Station	Beeville
PMN 10TX13 <sup>Y</sup>	236 a <sup>Z</sup>	370 a	556 a
Merkeron <sup>X</sup>	188 ab	77 b	145 b
PEPU 09FL03 <sup>W</sup>	271 a	65 b	143 b
PEPU 09FL01 <sup>W</sup>	153 ab	33 b	116 b
BMR sorghum <sup>V</sup>	74 ab	13 b	44 b
SX-17 <sup>U</sup>	111 ab	17 b	99 b
BMR pearl millet <sup>T</sup>	53 ab	3 b	3 b
PSH 09TX15 <sup>S</sup>	84 ab	13 b	89 b
Alamo switchgrass	87 ab	29 b	55 b
Tropical Isle Sunn Hemp	198 ab	81 b	-
Giant miscanthus (Mxg)	24 b	8 b	12 b
Energy cane <sup>R</sup>	248 a	50 b	86 b

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Feedstocks were ranked based on their overall suitability for utilization in an integrated biorefinery with the following conversion stages: 1) bioethanol from cellulose and hemicellulose fractions, 2) succinate from the liquid fraction prior to ethanol fermentation, 3) biopower from the residual lignin fraction, and 4) biosilica from the remaining ash fraction (Figure 1).

Figure 1. Illustration of conceptual integrated biorefinery producing ethanol, biopower, succinate, and silica.



The PMN had the highest overall ranking across all locations (Table 9). At Stephenville, PEPU 09FL03 and energy cane had the same ranking as the PMN,



followed by Merkeron and PEPU 09FL01 (Table 9a). At College Station and Beeville, napiergrasses and energy cane also ranked higher than other entries (Table 9b, 9c).

Table 9. Summary ranking of potential biorefinery (bioethanol, biopower, succinate, biosilica) across twelve candidate feedstocks. All traits were ranked based on their yield (kg ha<sup>-1</sup>). The feedstock entry that had the highest yield was ranked first for cellulose, hemicellulose, succinate and silica yield. However, the feedstock entry that had the lowest yield was ranked first for lignin yield.

a. Stephenville

Entry	Biorefinery rank					Overall
	Cellulose <sup>Z</sup>	Hemicellulose	Lignin	Succinate	Si	
PMN 10TX13 <sup>Y</sup>	2	3	10	3	1	1
Merkeron <sup>X</sup>	5	4	8	5	4	4
PEPU 09FL03 <sup>W</sup>	3	2	11	1	2	1
PEPU 09FL01 <sup>W</sup>	6	7	7	6	5	5
BMR sorghum <sup>V</sup>	10	10	3	10	8	10
SX-17 <sup>U</sup>	7	6	6	7	6	6
BMR pearl millet <sup>T</sup>	11	11	1	11	10	11
PSH 09TX15 <sup>S</sup>	8	9	5	9	7	8
Alamo switchgrass	9	8	4	8	9	8
Tropical Isle Sunn Hemp	1	5	12	4	12	7
Giant miscanthus (Mxg)	12	12	2	12	11	12
Energy cane <sup>R</sup>	4	1	9	2	3	1

<sup>Z</sup> All the traits were ranked based on their yield (kg ha<sup>-1</sup>). The feedstock entry that had the highest yield was ranked first for cellulose, hemicellulose, succinate and silica yield. However, the feedstock entry that had the lowest yield was ranked first for lignin yield. The feedstock entry with the smallest total was ranked first overall. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

Table 9. Continued

## b. College Station

Entry	Biorefinery rank					Overall
	Cellulose <sup>Z</sup>	Hemicellulose	Lignin	Succinate	Si	
PMN 10TX13 <sup>Y</sup>	1	1	12	1	1	1
Merkeron <sup>X</sup>	3	2	10	3	2	2
PEPU 09FL03 <sup>W</sup>	5	5	9	4	4	4
PEPU 09FL01 <sup>W</sup>	6	7	6	6	5	5
BMR sorghum <sup>V</sup>	10	10	2	10	6	8
SX-17 <sup>U</sup>	8	8	5	8	9	8
BMR pearl millet <sup>T</sup>	12	12	1	12	12	12
PSH 09TX15 <sup>S</sup>	9	9	4	9	7	8
Alamo switchgrass	7	6	7	7	8	7
Tropical Isle Sunn Hemp	2	4	11	2	11	5
Giant miscanthus (Mxg)	11	11	3	11	10	11
Energy cane <sup>R</sup>	4	3	8	5	3	3

## c. Beeville

Entry	Biorefinery rank					Overall
	Cellulose <sup>Z</sup>	Hemicellulose	Lignin	Succinate	Si	
PMN 10TX13 <sup>Y</sup>	1	1	11	1	1	1
Merkeron <sup>X</sup>	2	2	8	2	2	2
PEPU 09FL03 <sup>W</sup>	3	3	10	3	3	3
PEPU 09FL01 <sup>W</sup>	5	6	9	4	6	5
BMR sorghum <sup>V</sup>	8	9	3	9	8	8
SX-17 <sup>U</sup>	6	5	7	5	7	5
BMR pearl millet <sup>T</sup>	11	11	1	11	11	11
PSH 09TX15 <sup>S</sup>	7	7	5	6	5	5
Alamo switchgrass	9	8	4	8	9	9
Tropical Isle Sunn Hemp						
Giant miscanthus (Mxg)	10	10	2	10	10	10
Energy cane <sup>R</sup>	4	4	6	7	4	4

<sup>Z</sup> All the traits were ranked based on their yield (kg ha<sup>-1</sup>). The feedstock entry that had the highest yield was ranked first for cellulose, hemicellulose, succinate and silica yield. However, the feedstock entry that had the lowest yield was ranked first for lignin yield. The feedstock entry with the smallest total was ranked first overall. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

## Greenhouse evaluation

### *Statistical analysis*

Neither irrigation level main factor treatment nor interactions between irrigation level and feedstock entries were measured for biomass yield. This may indicate that sufficient drought stress was not achieved. There were no differences identified at irrigation level for any trait evaluated except NDF (Table 10). However, differences were found among feedstock entries for every trait.

### *Chemical composition*

Under deficit watering, PMN and perennial sorghum had the highest NDF concentration (Table 11a). However, the highest NDF content occurred in switchgrass.

The PMN and Merkeron had the highest succinate concentration under deficit watering, while the non-BMR annual sorghum had the lowest succinate concentration (Table 11a). Under well-watering, Merkeron, PEPU 09FL03, PEPU 09FL01 and switchgrass had the highest succinate concentration, the BMR annual sorghum had the lowest succinate concentration (Table 11b).

Table 10. Analysis of variance of biomass yield, hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF), crude protein (CP), and succinate concentration for greenhouse experiment.

	Biomass	Hemicellulose	Cellulose	ADL	NDF	CP	Succinate
water	ns <sup>Z</sup>	ns	ns	ns	*	ns	ns
trt	***	***	***	***	***	***	***
water*trt	ns	ns	ns	ns	ns	ns	**

<sup>Z</sup> NS (nonsignificant) or significant at  $P \leq 0.05$  (\*), 0.01 (\*\*), or 0.001 (\*\*\*).

Table 11. Greenhouse sample traits of biomass yield (g) and chemical composition including hemicellulose, cellulose, acid detergent lignin (ADL), neutral detergent fiber (NDF) (g kg<sup>-1</sup> biomass), crude protein (CP) (%), and succinate (g kg<sup>-1</sup> leaf).

a. deficit watering

Entry	Means						
	DM	Hemicellulose	Cellulose	ADL	NDF	CP	Succinate
	g	g kg <sup>-1</sup>				%	g kg <sup>-1</sup>
PMN 10TX13 <sup>Y</sup>	115.7 b <sup>Z</sup>	261 abcd	392 bc	53 bc	706 a	3 ab	11.1 a
Merkeron <sup>X</sup>	65 b	219 d	373 bcd	62 b	654 abc	2.96 ab	10.1 a
PEPU 09FL03 <sup>W</sup>	78 b	249 abcd	365 bcde	73 ab	687 ab	2.64 ab	9.4 ab
PEPU 09FL01 <sup>W</sup>	84.1 b	240 bcd	353 cdef	90 a	683 ab	1.93 b	9.3 ab
BMR Sorghum <sup>V</sup>	25.5 b	237 bcd	307 fg	16 e	560 d	2.71 ab	9.5 ab
SX-17 <sup>U</sup>	77.1 b	229 d	320 efg	26 de	575 d	1.93 b	1.5 c
BMR pearl millet <sup>T</sup>	17 b	276 ab	323 defg	28 cde	628 bcd	3.69 ab	10.2 abc
PSH 09TX15 <sup>S</sup>	50.7 b	280 ab	405 b	27 de	712 a	1.79 b	3.1 bc
Alamo switchgrass	4.5 b	306 a	280 g	61 abcd	647 abcd	3.67 ab	8.2 abc
Tropical isle Sunn Hemp	46.6 b	110 e	464 a	93 a	667 ab	5.74 a	4.6 abc
Giant miscanthus (Mxg)	18.2 b	273 abc	325 defg	60 b	658 ab	3.49 ab	4.5 abc
Energy cane <sup>R</sup>	236.4 a	231 cd	314 fg	35 cde	580 cd	2.11 ab	4.3 abc
grand mean	68.2						7.1

b. well watered

Entry	Means						
	Biomass	Hemicellulose	Cellulose	ADL	NDF	CP	Succinate
	g	g kg <sup>-1</sup>				%	g kg <sup>-1</sup>
PMN 10TX13 <sup>Y</sup>	142.2 ab <sup>Z</sup>	255 a	391 b	51 cd	697 ab	2.09 b	8.0 ab
Merkeron <sup>X</sup>	50 bc	235 a	380 bcd	70 abc	685 abc	3.51 ab	9.9 a
PEPU 09FL03 <sup>W</sup>	64.2 bc	269 a	322 cde	67 bc	657 abcd	2.34 b	11.1 a
PEPU 09FL01 <sup>W</sup>	74.3 bc	253 a	348 bcde	85 ab	686 abc	2.3 b	10.9 a
BMR Sorghum <sup>V</sup>	38.9 bc	261 a	306 e	18 e	585 d	2.08 b	0.8 b
SX-17 <sup>U</sup>	133.4 abc	249 a	319 cde	32 de	600 cd	2.58 b	9.0 ab
BMR pearl millet <sup>T</sup>	15 bc	282 a	319 cde	35 de	637 abcd	2.93 ab	
PSH 09TX15 <sup>S</sup>	56.7 bc	275 a	386 bc	31 de	692 abc	1.78 b	6.9 ab
Alamo switchgrass	3.9 c	323 a	350 bcde	91 ab	764 a	2.83 ab	10.4 a
Tropical isle Sunn Hemp	30.6 bc	96 b	463 a	97 a	656 abcd	5.25 a	6.9 ab
Giant miscanthus (Mxg)	10.3 bc	294 a	326 bcde	73 abc	692 abc	3.03 ab	5.3 ab
Energy cane <sup>R</sup>	227 a	253 a	315 de	35 de	602 bcd	2.25 b	7.8 ab
grand mean	70.5						7.9

<sup>Z</sup> Means within a column under each main factor followed by the same letter are not significantly different according to All Pairs, Turkey HSD. <sup>Y</sup> Pearl millet napiergrass hybrid PMN 10TX13. <sup>X</sup> Napiergrass cultivar Merkeron. <sup>W</sup> Napiergrass accession. <sup>V</sup> SDH2942 BMR sorghum. <sup>U</sup> Annual sorghum SX-17 cultivar. <sup>T</sup> Exceed BMR pearl millet. <sup>S</sup> Perennial sorghum hybrid PSH 09TX15. <sup>R</sup> Energy cane unknown accession.

## CONCLUSIONS

The PMN, napiergrass and energy cane entries had constantly higher biomass yield across all locations. At the two locations sunn hemp was included, it was roughly equivalent to the grass entries. Noting the importance of cellulose and hemicellulose fractions towards ethanol conversion, PMN, napiergrass, energy cane and sunn hemp had superior performance. Due to the lignin fraction's inhibition of bioethanol conversion, compare to the smaller benefit of lignin towards biopower production from bagasse, sunn hemp may not be an ideal feedstock for the proposed biorefinery. For biosilica and succinate, the highest yields were also found in PMN, napiergrass and energy cane. Sunn hemp was superior for succinate production; however, its silica yield was low. Total biomass yield was more important than concentration of measured components. Thus, PMN, napiergrass and energy cane appear to have the highest potential for utilization in the proposed biorefinery.

## REFERENCES

- [1] J. Hill, E. Nelson, D. Tilman, S. Polasky, D. Tiffany, Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels, *Proceedings of the National Academy of sciences* 103(30) (2006) 11206-11210.
- [2] M. Wang, J. Han, J.B. Dunn, H. Cai, A. Elgowainy, Well-to-wheels energy use and greenhouse gas emissions of ethanol from corn, sugarcane and cellulosic biomass for US use, *Environmental Research Letters* 7(4) (2012) 045905.
- [3] Renewable Fuel Association, World Fuel Ethanol Production.  
<<http://www.ethanolrfa.org/resources/industry/statistics/#1454099103927-61e598f7-7643>>, 2017 (accessed 28 April.2017).
- [4] A. Demirbas, Producing and using bioethanol as an automotive fuel, *Energy Sources, Part B* 2(4) (2007) 391-401.
- [5] H. Chen, *Biotechnology of Lignocellulose*, Springer Netherlands, 2014.
- [6] A. Amore, P.N. Ciesielski, C.-Y. Lin, D. Salvachúa, V.S. i Nogué Development of Lignocellulosic Biorefinery Technologies: Recent Advances and Current Challenges, *Australian Journal of Chemistry* 69(11) (2016) 1201-1218.
- [7] F. Cherubini, The biorefinery concept: using biomass instead of oil for producing energy and chemicals, *Energy Conversion and Management* 51(7) (2010) 1412-1421.
- [8] Energy Information Administration, Biofuels in the U.S. Transportation Sector.  
<[https://www.epw.senate.gov/public/\\_cache/files/b7674727-6eeb-4921-a4fb-6caaaea8d8a5/eiaethanolbushelsperacredata.pdf](https://www.epw.senate.gov/public/_cache/files/b7674727-6eeb-4921-a4fb-6caaaea8d8a5/eiaethanolbushelsperacredata.pdf)>, 2007 (accessed 21 June.2017).

- [9] Environmental Protection Agency, Overview for Renewable Fuel Standard. <<https://www.epa.gov/renewable-fuel-standard-program/overview-renewable-fuel-standard>>, (accessed 21 June.2017).
- [10] Environmental Protection Agency, Final Renewable Fuel Standards for 2017, and the Biomass-Based Diesel Volume for 2018. <<https://www.epa.gov/renewable-fuel-standard-program/final-renewable-fuel-standards-2017-and-biomass-based-diesel-volume>>, (accessed 21 June.2017).
- [11] J. Plank, Applications of biopolymers and other biotechnological products in building materials, Applied microbiology and biotechnology 66(1) (2004) 1-9.
- [12] T. Werpy, G. Petersen, A. Aden, J. Bozell, J. Holladay, J. White, A. Manheim, D. Eliot, L. Lasure, S. Jones, Top value added chemicals from biomass. Volume 1-Results of screening for potential candidates from sugars and synthesis gas, DTIC Document, 2004.
- [13] J.M. Pinazo, M.E. Domine, V. Parvulescu, F. Petru, Sustainability metrics for succinic acid production: a comparison between biomass-based and petrochemical routes, Catalysis Today 239 (2015) 17-24.
- [14] M.F. Bradfield, A. Mohagheghi, D. Salvachúa, H. Smith, B.A. Black, N. Dowe, G.T. Beckham, W. Nicol, Continuous succinic acid production by *Actinobacillus succinogenes* on xylose-enriched hydrolysate, Biotechnology for biofuels 8(1) (2015) 181.



- [15] H. Du, Z. Wang, W. Yu, B. Huang, Metabolic responses of hybrid bermudagrass to short-term and long-term drought stress, *Journal of the American Society for Horticultural Science* 137(6) (2012) 411-420.
- [16] U. Kalapathy, A. Proctor, J. Shultz, A simple method for production of pure silica from rice hull ash, *Bioresource Technology* 73(3) (2000) 257-262.
- [17] H.A. Currie, C.C. Perry, Silica in plants: biological, biochemical and chemical studies, *Annals of botany* 100(7) (2007) 1383-1389.
- [18] P. Van Soest, Rice straw, the role of silica and treatments to improve quality, *Animal Feed Science and Technology* 130(3) (2006) 137-171.
- [19] M.A. Lima, L.D. Gomez, C.G. Steele-King, R. Simister, O.D. Bernardinelli, M.A. Carvalho, C.A. Rezende, C.A. Labate, S.J. McQueen-Mason, I. Polikarpov, Evaluating the composition and processing potential of novel sources of Brazilian biomass for sustainable biorenewables production, *Biotechnology for biofuels* 7(1) (2014) 10.
- [20] R. Herrera, D. Fortes, M. Garc ía, A.M. Cruz, A. Romero, Study of the mineral composition in varieties of *Pennisetum purpureum*, *Cuban J. Agric. Sci* 42 (2008) 393.
- [21] V. Kindomihou, P.J. Meerts, R. Kjelgren, A.B. Sinsin, Effect of moisture stress on leaf silicification of three tropical fodder grass species (*Pennisetum purpureum*, *Panicum maximum* cv. C1 and *P. maximum* Jacq.) in Republic of Benin (West Africa), *American-Eurasian Journal of Agricultural and Environmental Sciences* 8(5) (2010) 530-537.

- [22] N. Srisittipokakun, N. Sangwaranatee, Comparative Study of Physical and Optical Properties of Glasses from SiO<sub>2</sub> and Napier Ash, *Advanced Materials Research*, Trans Tech Publ, 2014, pp. 401-404.
- [23] A.M.F.D. da Silva, K.A. Sales, V.S. Candido, S.N. Monteiro, C.M.F. Vieira, Characterization of Elephant Grass Ash as a Potential Clay Ceramic Addition, *Materials Science Forum*, Trans Tech Publ, 2015, pp. 585-590.
- [24] K.P. Woli, M.B. David, J. Tsai, T.B. Voigt, R.G. Darmody, C.A. Mitchell, Evaluating silicon concentrations in biofuel feedstock crops *Miscanthus* and switchgrass, *Biomass and Bioenergy* 35(7) (2011) 2807-2813.
- [25] K. Minu, K.K. Jiby, V. Kishore, Isolation and purification of lignin and silica from the black liquor generated during the production of bioethanol from rice straw, *Biomass and Bioenergy* 39 (2012) 210-217.
- [26] W. Hanna, C. Chaparro, B. Mathews, J. Burns, L. Sollenberger, J. Carpenter, *Perennial pennisetums*, American society of agronomy monograph series (2004).
- [27] A. Bogdan, *Tropical pasture and fodder plants*, Longman.1977.
- [28] J.E. Knoll, W.F. Anderson, Vegetative propagation of napiergrass and energycane for biomass production in the southeastern United States, *Agronomy journal* 104(2) (2012) 518-522.
- [29] K. Woodard, G. Prine, Forage yield and nutritive value of elephantgrass as affected by harvest frequency and genotype, *Agronomy journal* 83(3) (1991) 541-546.
- [30] S. Schank, D. Chynoweth, C. Turick, P. Mendoza, Napiergrass genotypes and plant parts for biomass energy, *Biomass and Bioenergy* 4(1) (1993) 1-7.

- [31] J.E. Knoll, W.F. Anderson, T.C. Strickland, R.K. Hubbard, R. Malik, Low-input production of biomass from perennial grasses in the coastal plain of Georgia, USA, *Bioenergy Research* 5(1) (2012) 206-214.
- [32] K. Rengsirikul, Y. Ishii, K. Kangvansaichol, P. Sripichitt, V. Punsuvon, P. Vaithanomsat, G. Nakamane, S. Tudsri, Biomass yield, chemical composition and potential ethanol yields of 8 cultivars of napiergrass (*Pennisetum purpureum* Schumach.) harvested 3-monthly in central Thailand, *Journal of Sustainable Bioenergy Systems* 3(02) (2013) 107.
- [33] C.D. Dowling, B.L. Burson, J.L. Foster, L. Tarpley, R.W. Jessup, Confirmation of pearl millet-napiergrass hybrids using EST-derived simple sequence repeat (SSR) markers, (2013).
- [34] R. Osgood, W. Hanna, T. Tew, Hybrid seed production of pearl millet  $\times$  napiergrass triploid hybrids, *Crop science* 37(3) (1997) 998-999.
- [35] R.W. Jessup, C.D. Dowling, "Seeded-yet-Sterile" Perennial Grasses: Towards Sustainable and Non-invasive Biofuel Feedstocks, *Bioenergy and Biological Invasions: Ecological, Agronomic and Policy Perspectives on Minimizing Risk* 5 (2015) 97.
- [36] K. Woodard, G. Prine, Dry matter accumulation of elephantgrass, energycane, and elephantmillet in a subtropical climate, *Crop science* 33(4) (1993) 818-824.
- [37] J.R. Harlan, J.M. de Wet, Toward a rational classification of cultivated plants, *Taxon* (1971) 509-517.
- [38] W.W. Hanna, D.D. Baltensperger, A. Seetharam, Pearl millet and other millets, *Warm-season (C4) grasses (warmseasonc4gra)* (2004) 537-560.

- [39] G.W. Burton, Breeding pearl millet, Plant breeding reviews, Springer 1983, pp. 162-182.
- [40] M.R. Jahansouza, R.K. Afshar, H. Heidari, M. Hashemi, Evaluation of yield and quality of sorghum and millet as alternative forage crops to corn under normal and deficit irrigation regimes, Jordan Journal of Agricultural Sciences 10(4) (2014).
- [41] D.J. Parrish, J.H. Fike, The biology and agronomy of switchgrass for biofuels, BPTS 24(5-6) (2005) 423-459.
- [42] J. Kiniry, K. Cassida, M. Hussey, J. Muir, W. Ocumpaugh, J. Read, R. Reed, M. Sanderson, B. Venuto, J. Williams, Switchgrass simulation by the ALMANAC model at diverse sites in the southern US, Biomass and bioenergy 29(6) (2005) 419-425.
- [43] L. Wright, Historical perspective on how and why switchgrass was selected as a “model” high-potential energy crop, ORNL/TM-2007/109 Oak Ridge, TN: Bioenergy Resources and Engineering Systems (2007).
- [44] S. McLaughlin, New switchgrass biofuels research program for the southeast, Oak Ridge National Lab., TN (United States), 1992.
- [45] M. Sanderson, R. Reed, S. McLaughlin, S. Wulschleger, B. Conger, D. Parrish, D. Wolf, C. Taliaferro, A. Hopkins, W. Ocumpaugh, Switchgrass as a sustainable bioenergy crop, Bioresource Technology 56(1) (1996) 83-93.
- [46] J.P. Muir, M.A. Sanderson, W.R. Ocumpaugh, R.M. Jones, R.L. Reed, Biomass production of ‘Alamo’ switchgrass in response to nitrogen, phosphorus, and row spacing, Agronomy Journal 93(4) (2001) 896-901.

- [47] J.A. Guretzky, J.T. Biermacher, B.J. Cook, M.K. Kering, J. Mosali, Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition, *Plant and Soil* 339(1-2) (2011) 69-81.
- [48] E. Heaton, T. Voigt, S.P. Long, A quantitative review comparing the yields of two candidate C 4 perennial biomass crops in relation to nitrogen, temperature and water, *Biomass and bioenergy* 27(1) (2004) 21-30.
- [49] R. Pyter, T. Voigt, E. Heaton, F. Dohleman, S. Long, Growing giant miscanthus in Illinois, University of Illinois Extension, Urbana-Champaign (2007).
- [50] E.A. Heaton, F.G. Dohleman, S.P. Long, Meeting US biofuel goals with less land: the potential of Miscanthus, *Global change biology* 14(9) (2008) 2000-2014.
- [51] L.G. Angelini, L. Ceccarini, N.N. o Di Nasso, E. Bonari, Comparison of *Arundo donax* L. and *Miscanthus x giganteus* in a long-term field experiment in Central Italy: analysis of productive characteristics and energy balance, *Biomass and bioenergy* 33(4) (2009) 635-643.
- [52] R. Pyter, E. Heaton, F. Dohleman, T. Voigt, S. Long, Agronomic experiences with *Miscanthus x giganteus* in Illinois, USA, *Biofuels: Methods and protocols* (2009) 41-52.
- [53] J.R. Kiniry, M.-V.V. Johnson, S.B. Bruckerhoff, J.U. Kaiser, R. Cordsiemon, R.D. Harmel, Clash of the titans: comparing productivity via radiation use efficiency for two grass giants of the biofuel field, *BioEnergy Research* 5(1) (2012) 41-48.
- [54] M. Kering, T. Butler, J. Biermacher, J.A. Guretzky, Biomass yield and nutrient removal rates of perennial grasses under nitrogen fertilization, *Bioenergy Research* 5(1) (2012) 61-70.

- [55] I.E. Palmer, R.J. Gehl, T.G. Ranney, D. Touchell, N. George, Biomass yield, nitrogen response, and nutrient uptake of perennial bioenergy grasses in North Carolina, *Biomass and Bioenergy* 63 (2014) 218-228.
- [56] B. Legendre, D. Burner, Biomass production of sugarcane cultivars and early-generation hybrids, *Biomass and Bioenergy* 8(2) (1995) 55-61.
- [57] S. Matsuoka, A.J. Kennedy, E.G.D.d. Santos, A.L. Tomazela, L.C.S. Rubio, Energy cane: its concept, development, characteristics, and prospects, *Advances in Botany* 2014 (2014).
- [58] J.E. Knoll, W.F. Anderson, E.P. Richard, J. Doran-Peterson, B. Baldwin, A.L. Hale, R.P. Viator, Harvest date effects on biomass quality and ethanol yield of new energycane (*Saccharum* hyb.) genotypes in the Southeast USA, *biomass and bioenergy* 56 (2013) 147-156.
- [59] M.E. Salassi, K. Brown, B.M. Hilbun, M.A. Deliberto, K.A. Gravois, T.B. Mark, L.L. Falconer, Farm-scale cost of producing perennial energy cane as a biofuel feedstock, *BioEnergy Research* 7(2) (2014) 609-619.
- [60] S. Shields, R. Boopathy, Ethanol production from lignocellulosic biomass of energy cane, *International Biodeterioration & Biodegradation* 65(1) (2011) 142-146.
- [61] J.R. Fedenko, J.E. Erickson, K.R. Woodard, L.E. Sollenberger, J.M. Vendramini, R.A. Gilbert, Z.R. Helsel, G.F. Peter, Biomass production and composition of perennial grasses grown for bioenergy in a subtropical climate across Florida, USA, *Bioenergy research* 6(3) (2013) 1082-1093.

- [62] C.-I. Na, L.E. Sollenberger, J.E. Erickson, K.R. Woodard, J.M. Vendramini, M.L. Silveira, Management of perennial warm-season bioenergy grasses. I. Biomass harvested, nutrient removal, and persistence responses of elephantgrass and energycane to harvest frequency and timing, *Bioenergy Research* 8(2) (2015) 581-589.
- [63] W.L. Rooney, J. Blumenthal, B. Bean, J.E. Mullet, Designing sorghum as a dedicated bioenergy feedstock, *Biofuels, Bioproducts and Biorefining* 1(2) (2007) 147-157.
- [64] W. Anderson, G. Sarath, S. Edme, M. Casler, R. Mitchell, C. Tobias, A. Hale, S. Sattler, J. Knoll, Dedicated herbaceous biomass feedstock genetics and development, *BioEnergy Research* 9(2) (2016) 399-411.
- [65] J.F. Pedersen, W.L. Rooney, Sorghums, Warm-Season (C4) Grasses (warmseasonc4gra) (2004) 1057-1079.
- [66] K. Porter, J. Axtell, V. Lechtenberg, V. Colenbrander, Phenotype, fiber composition, and in vitro dry matter disappearance of chemically induced brown midrib (bmr) mutants of sorghum, *Crop Science* 18(2) (1978) 205-208.
- [67] B.S. Dien, G. Sarath, J.F. Pedersen, S.E. Sattler, H. Chen, D.L. Funnell-Harris, N.N. Nichols, M.A. Cotta, Improved sugar conversion and ethanol yield for forage sorghum (*Sorghum bicolor* L. Moench) lines with reduced lignin contents, *BioEnergy Research* 2(3) (2009) 153-164.
- [68] J. Propheter, S. Staggenborg, X. Wu, D. Wang, Performance of annual and perennial biofuel crops: yield during the first two years, *Agronomy Journal* 102(2) (2010) 806-814.

- [69] J. Bin, Utilization of Green manure for raising soil fertility in China, *Soil Science* 135(1) (1983) 65.
- [70] P.P. Rotar, R.J. Joy, 'Tropic Sun'Sunn Hemp; *Crotalaria juncea* L, (1983).
- [71] Z. Mansoer, D.W. Reeves, C. Wood, Suitability of sunn hemp as an alternative late-summer legume cover crop, *Soil Science Society of America Journal* 61(1) (1997) 246-253.
- [72] K.S. Balkcom, D.W. Reeves, Sunn-hemp utilized as a legume cover crop for corn production, *Agronomy journal* 97(1) (2005) 26-31.
- [73] H.H. Schomberg, N.L. Martini, J.C. Diaz-Perez, S.C. Phatak, K.S. Balkcom, H.L. Bhardwaj, Potential for using sunn hemp as a source of biomass and nitrogen for the Piedmont and Coastal Plain regions of the southeastern USA, *Agronomy journal* 99(6) (2007) 1448-1457.
- [74] S.R. Kamireddy, J. Li, S. Abbina, M. Berti, M. Tucker, Y. Ji, Converting forage sorghum and sunn hemp into biofuels through dilute acid pretreatment, *Industrial Crops and Products* 49 (2013) 598-609.
- [75] K.B. Cantrell, P.J. Bauer, K.S. Ro, Utilization of summer legumes as bioenergy feedstocks, *biomass and bioenergy* 34(12) (2010) 1961-1967.
- [76] P. Van Soest, J. Robertson, Systems of analysis for evaluating fibrous feeds, Workshop on Standardization of Analytical Methodology for Feeds, Ottawa (Canada), 12-14 Mar 1979, IDRC, 1980.



- [77] P. Van Soest, J. Robertson, B. Lewis, Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle, *Journal of Dairy Science* 74(10) (1991) 3583-3597.
- [78] Y.H. Li, S.H. Woo, D.H. Choi, E.-H. Cho, Succinate causes  $\alpha$ -SMA production through GPR91 activation in hepatic stellate cells, *Biochemical and biophysical research communications* 463(4) (2015) 853-858.
- [79] S. Reidinger, M.H. Ramsey, S.E. Hartley, Rapid and accurate analyses of silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer, *New Phytologist* 195(3) (2012) 699-706.