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High sugar Eastern gamagrass (*Tripsacum dactyloides* L) cultivars as potential biofuel feedstock

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Abstract

Maize (*Zea mays* L) is the leading biofuel crop in the United States. Production of ethanol from maize requires relatively high energy inputs from petroleum-based products. The net energy required for growing maize and consequent contribution to atmospheric CO_2 make using maize for ethanol production inefficient. Warm season perennial grasses have excellent potential as alternative biofuel feedstocks. However, one of the drawbacks of using these grasses is their high lignin content, which restricts breakdown of cellulose into carbohydrates and sugar for fermentation into ethanol. This results in low net ethanol yield, a major challenge for biofuel production from cellulosic feedstocks. Eastern gamagrass (*Tripsacum dactyloides* L) is a warm season native American grass related to maize. To evaluate potential of Eastern gamagrass and gamagrass-*Zea* recombinant lines as a biofuel feedstock, seven varieties were analyzed for their chemical composition and fermentable sugar production. Based on total sugar yield, gamagrass-*Zea* recombinant cultivars Eagle Point Devil Corn and Sun Devil reached 595.8 and 456.5 mg g^{-1} raw biomass, respectively. Compared to the other gamagrass varieties and energy crops switchgrass and bermudagrass, these gamagrass-Zea recombinants gave a significantly higher ($P<0.05$) sugar yield. DNA fingerprinting revealed Sun Devil contains introgressed *Zea* alleles at three loci associated with sugar synthesis. At theoretical yield of 386.1 liters of ethanol per ton of dry biomass, the Eagle Point Devil Corn cultivar has potential as a high yielding biofuel feedstock. The next step will be to conduct agronomic studies to evaluate field performance and biomass yield of this promising new feedstock.

Keywords: Eastern gamagrass, *Tripsacum*, biofuel, ethanol, energy crop, sugar

Introduction

Approximately 90% of our current global energy comes from non-renewable, fossil resources of petroleum, natural gas, and coal. Transitioning to renewable biofuels will help offset non-renewable fossil fuel depletion, provide sustainable energy sources for the future, and aid reduction in CO_2 emissions. Starch from maize grain is easily converted into ethanol and production of ethanol from maize has been steadily climbing since 2002. In the United States in 2011, 27.3% of the maize crop (~3 billion quintals) was used for ethanol production (NCGA 2012). In addition to being a first generation biofuel, maize is a primary global food and feed crop. Research that showed there is a negative net energy of -4.30 MJ per liter of ethanol from maize grain underscored production of ethanol from maize for transportation fuel is inefficient (Pimentel and Patzak, 2005). Concerns about use of an important food crop for fuel and inefficiency of the process are why we need to rapidly expand alternative cellulosic feedstocks and increase efficiency of the cellulose-glucose-ethanol conversion process.

Some warm season perennial grasses with high biomass yield that have potential as sustainable cellulosic feedstocks include switchgrass (*Panicum virgatum* L), giant miscanthus (*Miscanthus x giganteus*, a hybrid between *M. sinensis* and *M. sacchariflorus*), and bermudagrass [*Cynodon dactylon* (L) Pers.] (Xu et al, 2011; Keshwani and Cheng, 2009). Typical production of perennial grasses for biomass/biofuel requires much less petroleum-based chemical inputs than grain maize production. Many of these grasses can be grown on marginal land or abandoned fields not suitable for profitable production of food and feed crops. Identification and development of feedstock crops that will give high biomass yield and high sugar yield for conversion to ethanol will make commercial biofuel production from these crops feasible.

Compared with soluble sugar- or starch-based biofuels, "second-generation" cellulosic ethanol is a more promising alternative fuel with regard to carbon footprint and net energy (Lemus and Parrish, 2009). Among all the potential cellulosic feedstocks, dedicated energy crops have attracted much attention due to the volume of biomass they can provide on a sustainable basis. Although progress towards a cost-effective grass-to-ethanol conversion has been made, the commercialization of cellulosic ethanol based on these grasses has not been realized due to complex biomass composition and recalcitrant biomass structure. Since there is significant genetic

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diversity among different grasses, this substantially affects the biochemical conversion and sugar production capability of different feedstocks. Therefore, it behooves us to investigate potential of other under- or unstudied grasses as biofuel feedstock crops.

Eastern gamagrass (*Tripsacum dactyloides* L) is a perennial warm-season C4 grass native to the Americas. It is a wild relative of maize that has potential to produce large amounts of biomass in the southeastern United States. Some of its many desirable characteristics as an energy crop include high biomass yield, longevity of established fields for decades, adaptation to different soil and climate conditions, non-invasiveness, carbon sequestration capacity, soil phytoremediation ability, and easy integration into existing farming operations (Comis, 2005; Douglas, 2000; Grabowski et al, 2004; Hinchman et al, 1999; Mashingo et al, 2008; van der Grinten, 2007; Weimer and Springer, 2007). Gamagrass yields of up to 24,965 kg ha^{-1} (Owsley 2008) are comparable to those of switchgrass and bermudagrass (Grabowski et al, 2004; Parrish and Fike 2005; Hill et al, 1993). The high carbohydrate (i.e. cellulose and hemicellulose) content of gamagrass is an added advantage that warrants systematic investigation for its application as a new energy crop (Ge et al, 2012).

To evaluate the biofuel potential of gamagrass, seven varieties were subjected to compositional analysis and biochemical conversion for fermentable sugar production (Xu et al, 2012). Pretreatment with sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4), two of the most common biomass pretreatment techniques, were employed to improve the enzymatic digestibility of gamagrass. Pretreatment was followed by enzymatic hydrolysis of the pretreated biomass to determine sugar production. The best gamagrass variety was determined based on the release of total sugars during biomass conversion. The results were also compared with those of switchgrass and bermudagrass.

Materials and Methods

The seven gamagrass varieties that were analyzed for composition and sugar conversion included three endemic species and four gamagrass-*Zea* recombinants. The native species include two *Tripsacum dactyloides* accessions. One, referred to as “Door”, was collected by Eubanks at the Indiana University Hilltop Experiment Station in Bloomington, Monroe County, Indiana in 1985. It was originally collected by Lois Farquharson in Santa Claus, Spencer County, Indiana (Farquharson, 1955). The other one was collected by Eubanks at Eagle Point, Davis Bayou, Jackson County, Mississippi in 2002. Both are tetraploids (4n=72). The third endemic is *Tripsacum* sp. collected by Ervin Wilson in Nobogame, Sonora, Mexico. Its ploidy has not been determined.

Eubanks broke the sterility barrier between *Zea* and *Tripsacum* when she recovered fully fertile re-

Figure 1 - Sun Devil cultivar, a high sugar gamagrass-*Zea* recombinant with *Tripsacum*-like phenotype.

combinants from crosses between Eastern gamagrass (*Tripsacum dactyloides* L) and diploid perennial teosinte (*Zea diploperennis* Iltis, Doebley and Guzmán) (Eubanks, 2001, 2006). The hybrids provide a genetic bridge for introgressing *Tripsacum* genes into *Zea* and vice versa. The recombinant plants [(*Tripsacum dactyloides* x *Zea diploperennis*) x *Zea mays*)] are of two general phenotypes; some more closely resemble the *Zea* parent and others are more *Tripsacum*-like in appearance. Although the *Zea*-like recombinants are perennial, they are not currently being considered for biofuel feedstock since they do not survive for prolonged periods in temperatures below -2°C. The gamagrass-like recombinants are winter hardy. They die back after a hard freeze in the fall and put out new growth in the spring. They are also cross fertile with *Zea* species. The cultivars investigated in this study are Sun Devil, Devil Corn 1, Devil Corn 2, and Eagle Point Devil Corn. Sun Devil (Figure 1) is a cross between *Tripsacum dactyloides* from Indiana and a gamagrass-*Zea* recombinant referred to as Tripsacorn (Eubanks, 1992). The Tripsacorn pollen donor is a cross between a *T. dactyloides* female parent and *Z. diploperennis* pollen donor. Devil Corn 1 and Devil Corn 2 were derived from backcrosses of Sun Devil as the female parent with Tripsacorn as pollen donor. Eagle Point Devil Corn is a more complex recombinant derived by crossing the Eagle Point *T. dactyloides* from Mississippi with (7022 X Devil Corn), a hybrid between Tripsacorn and maize inbred line

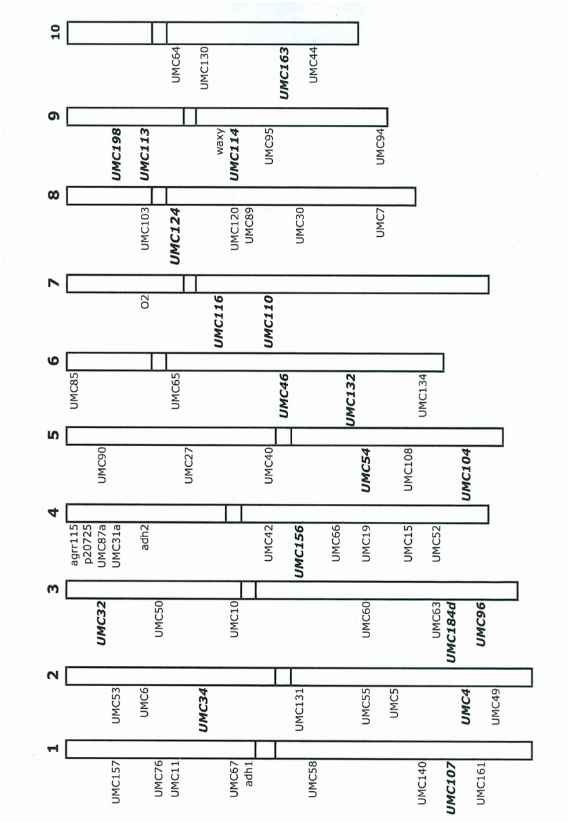

Figure 2 - Schematic of the 10 maize linkage groups showing positions of molecular marker loci probes used in the DNA fingerprinting. Probes italicized in bold are loci that show *Zea* introgression in Devil Corn. Ten Linkage Genetics proprietary RFLP probes are not included.

W64A. The 7022 X Devil Corn (2n=20) pollon donor is a cross between female parent 7022 and Devil Corn pollen donor. The 7022 female parent of that cross is a cross between Tripsacorn and maize inbred W64a as pollen donor. W64a is a non stiff stalk Reid's yellow dent maize. We harvested plant biomass of the seven varieties for compositional analysis in November 2011 from a nursery at the Duke University Lemur Center that was established in 2009. The November harvest was at the end of the growing season, which is not the optimal time for plant growth. To evaluate the impact of harvest time on composition and conversion results, we also harvested biomass from the Eagle Point Devil Corn cultivar at the end of July 2012. Post harvest plant material was dried in an oven at 50°C for 72 hours, then ground and stored at room temperature. The gamagrass screening, pretreatment optimization, composition, and conversion analytical methods have been previously detailed (Xu et al, 2012).

DNA fingerprinting was done to assess (or not) introgression of *Zea* genes into the gamagrass cultivars, the original *Zea* and *Tripsacum* parent plants, Tripsacorn, and Sun Devil. Fresh leaf tissue was harvested and shipped on dry ice by overnight delivery to Linkage Genetics in Salt Lake City, Utah for processing. The laboratory procedures followed standard restriction fragment length polymorphism (RFLP) genotyping protocol (Helentjaris et al, 1985, 1986). DNA was isolated, digested with the BamHI restriction enzyme, then transferred to Southern blots and probed with 75 mapped RFLP markers (Figure 2). Allelic bands on the autoradiographs were scored and entered into Excel files. The data were then analyzed to identify bands shared among Sun Devil, Tripsacorn, and the original *Tripsacum* and *Zea diploperennis* parents involved in the pedigrees of Sun Devil and Eagle Point Devil Corn. The Eagle Point Devil Corn was not included in the genotyping because the cross had not been made when the DNA fingerprinting was done. However, since Eagle Point Devil Corn is a derivative of Sun Devil and maize, we expect it shares many of the same Zea alleles.

Results

The structural carbohydrate (i.e. cellulose and hemicellulose) content of the different gamagrass varieties ranged from 57.2% to 64.8% (Xu et al, 2012). This is higher than switchgrass (53.5%) and bermudagrass (44.9%) (Table 1). The cultivar Eagle Point Devil Corn has a significantly ($P<0.05$) higher total carbohydrate content than the other varieties except for "Door", and it has the highest glucan content of all the varieties (Table 1). The glucan content of Eagle Point Devil Corn harvested in July is significantly ($P<0.0.5$) higher than that harvested in November. This makes July harvesting advantageous as glucose is a more favorable sugar substrate for ethanol fermentation. The total lignin (AIL+ASL) of gamagrass (22.9-26.4%) is higher than switchgrass (21.4%) and bermudagrass (19.3%). Since lignin hinders enzyme access to biomass carbohydrates for sugar production during hydrolysis, it is anticipated the higher lignin might be offset by higher carbohydrate levels when using gamagrass-*Zea* recombinants as a feedstock to produce fermentable sugars. The total lignin of Eagle Point Devil Corn harvested in July is higher than that harvested in November. This is probably because of the inclusion of seed spikes in the July harvest.

Sugar yields of grasses differ according to whether the pretreatment chemical is alkali or acid, chemical loading, reaction temperature, and residence time. The pretreatment conditions applied in this study were based on our experience with the pretreatment of other cellulosic biomass. Gamagrass was pretreated using 1% NaOH or 1% H_2SO_4 at 121°C for 60 min for improvement of enzymatic digestibility. The pretreated biomass was hydrolyzed enzymatically for sugar production. After pretreatment at 1% NaOH at 121°C for 60 min, the total sugar yield (sum of glucose, xylose, arabinose, and galactose) of gamagrass and gamagrass-*Zea* recombinant varieties in alkaline enzymatic hydrolysis ranged from 354.3 to

Table 1 - Composition of gamagrass and gamagrass-*Zea* recombinant varieties, switchgrass and bermudagrass.

	Components Dry Wt (%)							
Gamagrass variety	AIL	ASL	Ash	Glucan	Xylan	Arabinan	Galactan	Other
Devil Corn 1§	23.03 (0.41)	2.58 (0.05)	4.89 (0.08)	33.51 (1.72)	18.69 (0.67)	3.80 (0.20)	1.42 (0.08)	12.1 (2.70)
Devil Corn 2§	21.93 (1.21)	2.35 (0.04)	2.41 (0.16)	35.21 (0.51)	20.20 (0.67)	3.94 (0.14)	1.21 (0.03)	12.8 (0.53)
Sun Devil§	22.93 (0.92)	2.68 (0.10)	2.83 (0.13)	34.41 (2.19)	20.54 (1.71)	4.48 (0.10)	1.50 (0.08)	10.7 (0.59
Eagle Point	21.32 (0.64)	2.37 (0.06)	3.82 (0.31)	35.52 (1.46)	18.58 (1.13)	3.25 (0.15)	1.00 (0.04)	14.2 (2.47)
Eagle Point Devil Corn§ (November harvest)	20.77 (0.84)	2.14 (0.03)	2.39(0.40)	39.71 (0.91)	21.00 (0.53)	3.18 (0.19)	0.91 (0.09)	9.88 (1.67)
Eagle Point Devil Corn† (July harvest)	23.33 (0.71)	3.06 (0.42)	5.77 (0.00)	43.26 (1.13)	18.59 (0.75)	Not detected	Not detected	6.26 (2.36)
Hills Above Nobogame§	20.42 (0.77)	3.19 (0.11)	2.72 (0.20)	33.23 (1.04)	18.85 (0.61)	3.95 (0.42)	1.12 (0.12)	16.5 (2.52
Door§	20.19 (0.59)	2.70 (0.01)	1.91 (0.09)	35.60 (0.87)	20.78 (0.99)	4.42 (0.03)	1.26 (0.03)	13.1 (1.32)
Switchgrass‡	17	4.36	3.77	32	17.9	1.87	1.73	21.4
Bermudagrass∫	15.4	3.96	6.69	25.6	15.9	1.95	1.46	29.1

Values are averages of 3 replicates. Values in parenthesis represent standard deviations; AIL refers to acid insoluble lignin; ASL refers to acid soluble lignin
§Data from Xu et al, 2012; †Data from this study; ‡Data from Xu et al, 2010; ∫Data from Wang et al, 2010

595.8 mg g^{-1} raw biomass (Figure 3). Since glucose is the monomer of cellulose and xylose is the major building block of hemicelluloses, their yields were determined to evaluate the changes of cellulose and hemicelluloses, respectively, in biomass conversion, while arabinose and galactose were measured but not reported due to their low contents in the biomass. The glucose yields of gamagrass and gamagrass-*Zea* recombinant varieties in alkaline enzymatic hydrolysis ranged from 215.5 to 410.1 mg g^{-1} raw biomass. The xylose yields of different gamagrass varieties were comparable, ranging from 121.9 to 169.5 mg g^{-1} raw biomass. Compared with November harvest, the sugar yield, especially glucose yield, of Eagle Point Devil Corn harvested in July was much higher. This was because of the improved convertibility of carbohydrates as well as the higher glucan content of July harvest. After pretreatment at 121°C using 1% H_2SO_4 for 60 min, the total sugars recovered from the combined prehydrolysate and hydrolysate ranged from 350.4-465.3 mg g^{-1} raw biomass (Figure 3). The total sugar yields of Sun Devil and Hills Above Nobogame were significantly ($P<0.05$) higher than those of other gamagrass varieties, and no significant ($P<0.05$) difference in sugar production was found between them. After pretreatment and acid enzymatic hydrolysis, the yields of glucose and xylose ranged from 176.5 to 253.1 and 130.3 to 154.7 mg g^{-1} raw biomass, respectively (Xu et al, 2012).

Based on the above results, Eagle Point Devil Corn was recommended if NaOH pretreatment is applied. Our follow-up study (Xu et al, 2012) on pretreatment conditions showed applying 1% NaOH at 121°C for 60 min was sufficient to maximize glucose yield (595.8 mg g^{-1} raw biomass), at which the glucan conversion reached 85.3%. Similarly, 1% NaOH at 121°C for 60 min was sufficient to maximize xylose yield (169.5 mg g^{-1} raw biomass), at which the xylose conversion reached 80.2%. The change of total sugar yield with NaOH concentration and residence time was similar to those of glucose and xylose yields (Table 1). Therefore, it was recommended that 1% NaOH and 60 min be applied for NaOH pretreatment of Eagle Point Devil Corn for fermentable sugar production, at which the total sugar yield was 595.8 mg g^{-1} raw biomass, with a carbohydrate conversion of 83.8%. When the Eagle Point Devil Corn biomass was harvested in November after a freeze, the glucose yield was 298.5 mg g^{-1} raw biomass, the xylose yield was 159.9 mg g^{-1} raw biomass, and the total sugars were 479.6 mg g^{-1} raw biomass (Xu et al, 2012). In order to evaluate impact of late harvest versus optimal harvest time on sugar yield, we compared the glucose and xylose yields of Eagle Point Devil Corn biomass collected in November 2011 and that collected in July 2012, which was the optimum feedstock harvest time for biofuel production. Pretreated at best conditions, the glucose and xylose yields of plants harvested in July were 37.4% and 6% greater respectively, than plants harvested in November. Considering the comparable carbohydrate content between November and July harvest, the higher sugar yield of July harvest was apparently due to the higher susceptibility of plant biomass to biochemical conversion. These results show that plants should be harvested during their peak growing period for greatest efficiency of biomass conversion to ethanol. Ethanol yields are substantially reduced in biomass harvested at the end of the growing season.

Since the total sugar yield of Sun Devil is significantly ($P<0.05$) higher under H_2SO_4 pretreatment, Sun Devil was selected for H_2SO_4 pretreatment. Similarly, the combination of 1% H_2SO_4 at 121°C for 60 min was sufficient to maximize glucose yield in enzymatic hydrolysis (252.6 mg g^{-1} raw biomass) (Xu et al, 2012), at which an overall glucan conversion of 66.1% was reached after including the conversion achieved in pretreatment (Table 2). One percent H_2SO_4 and 60 min was also sufficient to maximize xylose yield in pretreatment (149.3 mg g^{-1} raw biomass), at which an overall xylan conversion of 64.0% was reached after the conversion achieved in enzymatic hydrolysis was included (Table 2). Based on total sugar yield, 1% H_2SO_4 and 60 min was recommended for H_2SO_4 pretreatment of Sun Devil for fermentable sugar production, at which the total sugar yield was 456.5 mg

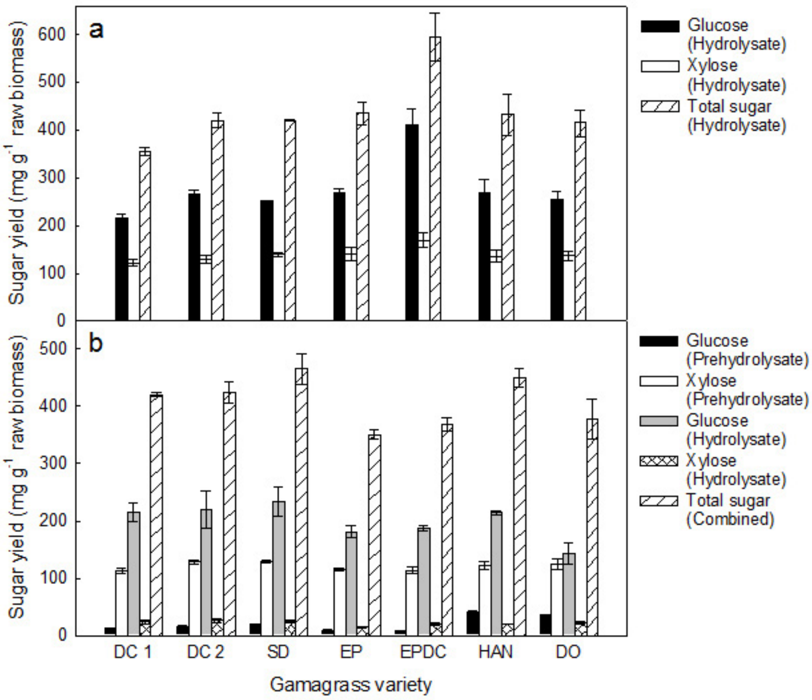

Figure 3 - Comparison of sugar yields of seven gamagrass and gamagrass-*Zea* recombinant varieties under different pretreatment conditions: a) 121°C, 1% NaOH, 60 min; b) 121°C, 1% H_2SO_4, 60 min. EPDC in a) was harvested at peak growth in July 2012, all other samples were harvested at the end of the growing season in November 2011.

g^{-1} raw biomass, with an overall carbohydrate conversion of 66.8%. Since the analysis of Eagle Point Devil Corn biomass harvested in July revealed higher sugar content, we expect the sugar yields for Devil Corn will be higher when harvested at the optimal time at peak summer growth.

Sun Devil DNA fingerprinting revealed visible bands at 41 (54.7%) of the 75 maize molecular marker loci. Twenty-three probes revealed bands shared between Sun Devil and *Zea diploperennis* at 23 molecular marker loci, confirming *Zea* introgression at these particular genetic loci (Figure 2). Three of the probes that revealed *Zea* introgression are associated with genes involved in sucrose synthesis. UMC113 and UMC114, which map to maize linkage group (i.e. chromosome) 9, are associated with the *Sh1* and *sus1* genes, respectively. UMC184d, which maps to maize linkage group 3, is associated with the *Sh2* gene. The *Sh1* gene encodes the sucrose synthase 1 (SS1) enzyme (Chourey and Nelson, 1979). Subunits of the SS1 protein react with the sucrose synthase 2 (SS2) isozyme, which is encoded by the *sus1* locus (Chourey et al, 1986). *Sh2* is involved in de-activation of ADP-glucose pyrophosphorylase, which causes accumulation of sucrose (Laughnan, 1953). Since Eagle Point Devil Corn is a derivative of Sun Devil and maize, it is expected the same *Zea* alleles and probably others are present in it. Since we did not measure sucrose in this study, we need to further investigate a possible link with the elevated glucose and xylose yields in the two high sugar gamagrass-*Zea* recombinants.

Discussion

Due to the higher carbohydrate content of the Eagle Point Devil Corn cultivar, its total sugar yield was higher than switchgrass and bermudagrass. Based on comparative results, it seems that although this cultivar does not necessarily give a higher carbohydrate conversion than other intensively studied herbaceous feedstocks during biochemical conversion, it is a competitive feedstock in terms of its total sugar production due to its high carbohydrate content. Because sugar yields can significantly increase the ethanol yield above other feedstocks, the Eagle Point Devil Corn cultivar deserves further investigation for its potential as a future biofuel crop.

To better evaluate the conversion of Eagle Point Devil Corn to fermentable sugars, comparisons of carbohydrate conversion and sugar yield were made with switchgrass and bermudagrass feedstocks (Table 2). After NaOH pretreatment at the best conditions (121°C, 1% NaOH, 60 min), the total sugar yield of Eagle Point Devil Corn reached 595.8 mg g^{-1} raw biomass, 83.8% of the theoretical yield, and total sugar yield exceeded NaOH-pretreated switchgrass and bermudagrass by 51.6% and 67.2%, respectively. After H_2SO_4 pretreatment at the best conditions (121°C, 1% H_2SO_4, 60 min), the total sugar yield of Sun Devil reached 456.5 mg g^{-1} raw biomass, 66.8%

Table 2 - Comparisons of carbohydrate conversion and sugar yield between gamagrass, switchgrass and bermudagrass biomass feedstocks.

Feedstock	Pretreatment conditions	Carbohydrate conversion (%)			Sugar yield (mg g^{-1} raw biomass)			Reference
		Glucan	Xylan	Total carbohydrates	Glucose	Xylose	Total sugars	
Switchgrass	121°C, 1% NaOH, 30 min	71.4	56.4	65.6	253.8	114.7	393.0	Xu et al (2010)
	150°C, 1% H_2SO_4, 10 min	75.8	62.8	67.3	337.8	157.6	495.4	Zhou et al (2012)
Bermuda grass	121°C, 0.75% NaOH, 30 min	90.4	65.1	70.8	257.1	117.5	356.3	Wang et al (2010)
	140°C, 1.2% H_2SO_4, 30 min	94.9	93.5	87.4	269.9	168.9	438.8	Redding et al (2011)
EPDC*	121°C, 1% NaOH, 60 min	85.3	80.2	83.8	410.1	169.5	595.8	This study
Sun Devil	121°C, 1% H_2SO_4, 60 min	66.1	64.0	66.8	252.6	149.3	456.5	Xu et al (2012)

*EPDC refers to Eagle Point Devil Corn

of the theoretical yield, 8% less than H_2SO_4-pretreated switchgrass but 10% higher than H_2SO_4-pretreated bermudagrass. It is noted that the Sun Devil biomass was harvested in November. When the data are normalized to accommodate the 24% increase in total sugar yield when harvested at the optimal growth period, the total sugar yield of Sun Devil is approximately 566.1 mg g^{-1} raw biomass, which is approximately 14.3% and 29% higher than H_2SO_4-pretreated switchgrass and bermudagrass, respectively. To get an idea of the practical impact of these differences in sugar conversions and yields on biofuel production, we calculated theoretical ethanol yields based on sugars that can be obtained by pretreatment and enzymatic hydrolysis (http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html). The ethanol yield for Eagle Point Devil Corn after NaOH pretreatment is 386.1 liters per ton dry biomass. Compared to the theoretical yield of switchgrass, which is 254.7 liters per ton dry biomass, there is a 51.6% boost in yield. Compared to the theoretical yield of bermudagrass, which is 61.0 gal ton^{-1} dry biomass, the boost in yield is 67.2%. In conclusion, the sugar yields of Eagle Point Devil Corn and Sun Devil exceed other potential energy crops, making these gamagrass-*Zea* cultivars promising feedstocks for biofuel development. Depending on pretreatment type, process conditions, solid and carbohydrate recovery after pretreatment, enzyme hydrolysis efficiency and fermentation efficiency (ability of microorganism to ferment both pentose (C5) and hexose (C6) sugars), the actual yield can vary from 60 to 90% of the estimated values. Therefore, it is important to balance process efficiency, productivity, and costs to maximize biofuel production using cellulosic feedstocks. Since our calculations are based on actual sugars extracted rather than raw feedstock biomass, the numbers more closely approximate actual amounts that can be expected.

Fifty-seven percent of the probes revealed introgression of *Zea* alleles in Sun Devil. Three introgressed *Zea* genes are associated with molecular markers mapped to regions for genes involved in sugar synthesis. These findings indicate it will be advantageous to employ a molecular marker-assisted breeding program to accelerate and enhance selection of high sugar gamagrass-*Zea* recombinants for future biofuel development.

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