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Evaluation of Brown-Midrib Genotypes for Fresh and Dry Biomass Suitable for Bio fuel Production

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Abstract: **Global economic development is highly dependent on fossil fuel supplies which are constrained not only by limited availability but also generate high levels of pollution. First generation ethanol comes from sugar crops such as sweet sorghum, sugarcane and corn. Conventional plant breeding methods are very useful to create substrate for second generation lignocellulosic bioenergy system. Improved brown midrib sorghum feedstock contains less lignin in their stems than the other sorghum types which results in increase in conversion efficiency. High biomass sorghum trait in bmr background can be achieved through introgression method. In present investigation brown midrib parent and their derivatives were compared for fresh and dry biomass yield. Brown midrib sorghum, is regarded as the most promising feedstock source for second generation ethanol production because of several advantages, high biomass yield, rapid growth, wide adaptability etc.**

1. INTRODUCTION

Sorghum is characterized by a vastly diverse germplasm in terms of phenotypic and morphological traits. Sorghums can be classified into four main groups depending on their production characteristics: grain sorghum, forage sorghum (FS), high-tonnage sorghum (energy), and sweet sorghum. Sorghums have generated interest as an alternative bioenergy feedstock since the 1970s. Although cellulosic biomass is receiving growing attention as a renewable feedstock, Lignocellulosic biomass is mainly composed of plant cell walls, with the structural carbohydrates cellulose and hemicellulose and heterogeneous phenolic polymer lignin as its primary components.

There is a growing interest for alternative energy sources because of the fossil fuel crises. Ethanol used as automotive fuel has increased at least six times in the current century. According to the Renewable Fuels Association, in 2010 the USA bio-refineries generated 13 billion gallons of bio-ethanol and the year before worldwide production reached 19 billion. This noteworthy increment is in its majority based on maize and sugar cane as raw materials (Berg, 2004; Renewable Fuels Association, 2010)

IEA (2010) provides a clear definition of first generation biofuels and second generation biofuels. Sorghum is a multipurpose crop with the potential to achieve sustainable biofuel production, human food and animal feed products. Typical first generation biofuels are sugarcane ethanol, starch-based or “corn” ethanol and biodiesel. Sugar rich crops especially those that yield multiple end products, are promising. Alternative energy sources that are cost effective and technologically sound need to be developed. First generation biofuels, like ethanol and fatty acids ethyl esters mixed with gasoline and diesel are used in several countries. The feedstock for producing first generation biofuels either consists of sugar, starch and oil crops or animal fats, which in most cases can also be used as food and feed or consists of food residues. Second generation biofuels are those biofuels produced from cellulose, hemicellulose or lignin. Examples of 2nd-generation biofuels are cellulosic ethanol and Fischer-Tropsch fuels. As the first generation biofuel feedstocks have created some concern regarding the food security and environmental issues there is a greater need in search of suitable feedstocks for the second generation biofuels.

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In recent years, introduction of sorghum plants containing the bmr gene generated much interest because plants with this trait have lower lignin concentrations than conventional types. The BMR mutant of forage sorghum contained substantially less cell wall content than other sorghum types and resulted in greater fiber digestibility. The enhanced cell wall digestibility from BMR sorghum improved milk yield of mid-lactation dairy cows at grazing. The reduced levels of lignin increase the bioconversion efficiency of biomass and reduce the cost of production of biofuels.

Biomass energy has the potential to greatly reduce our greenhouse gas emissions. Biomass creates about the same amount of carbon dioxide as fossil fuels, but every time a new plant grows, carbon dioxide is actually removed from the atmosphere. The net emission of CO2 will be zero as long as plants continue to be replenished for biomass energy purposes.

These energy crops, such as fast-growing trees and grasses, are called biomass feed-stocks. The use of biomass feed-stocks can also help increase profits for the agricultural industry. Agro-industrial biomass comprised on lignocellulosic waste is an inexpensive, renewable, abundant and provides a unique natural resource for large-scale and cost-effective bio-energy collection(Zahid Anwar et.al 2014) Dedicated biomass crops are typically non-food crops grown as feed-stocks for the purpose of transportation fuel, energy production and a wide range of industrial end uses. Instead of crop residue, dedicated biomass crops such as Sorghum Miscanthus and switchgrass among others, could be used for bioenergy production since both species have been reported to produce high yields of biomass with relatively low nutrient. Plant biomass is the most important trait because of abundant amount of Cellulose which can be converted into monomeric sugars. Significant (P B 0.05) differences were observed for fresh biomass and stalk yields. Fresh biomass varied from 39.0 to 67.0 t ha-1 with a mean of 58.0 t ha-1 across the locations. The greater biomass production by test hybrids over test OPVs indicated the expression of heterosis for biomass production SS Rao et.al (2013).Presently second generation lingo cellulosic biomass and agricultural residues is potential advantage for bio-ethanol production.Lynd et al., 1991; Sánchez and Cardona, 2008; Sims et al., 2010.Recentlylignocellulosic biomasses have gained increasing research interests and special importance because of their renewable nature (Asgher, Ahmad, & Iqbal, 2013; Ofori-Boateng & Lee, 2013)

2. FEED STOCKS

Feed stocks can be derived from any cellulosic source like agricultural, forest, or municipal waste products. Biomass can come in almost any form. Typical feed stocks include forest residues, agricultural field crops and residues, wood and wood residues, aquatic plants (algae), and fast-growing trees and plants. Typical waste feed stocks include municipal solid waste, construction waste, agricultural waste, animal manure, food processing waste,waste cooking oil, paper mill residue and wastewater treatment sludge. The process through which the biomass is converted to energy is dependent on the chemical composition, homogeneity, size, amount, and water content of the feedstock.

3. CONVERSION PROCESS

Ethanol can be produced from variety of carbohydrates (mono, di or polysaccharides. The most common monosaccharides are pentoses (n=5 and hexoses(n=6,glucose).Glucose is most common sugar in animal system. In plants sugar is transported in the form of disaccharides (sucrose, maltose and lactose).Starch and cellulose are polymers composed of glucose. Polysaccharide decompose into disaccharides and monosaccharide and converted into ethanol.

Cellulose ⟹ **Glucose** ⟹ **Ethanol**

Cellulose is the abundant polymer in nature and can be depolymerise into monomeric sugar for ethanol recovery**.** Most of the cellulosic biomass comes from forest. Field crops are advantageous in developing countries because these crops fulfil food grain requirement apart from cellulosic source.

4. MATERIAL & METHODS

The experiment was carried out at Directorate of Sorghum Research farm consisting of eighteen brown midrib sorghum genotypes along were sowed in randomized block design.

Land preparation and manuring: Two ploughings followed by leveling for good soil tilth. Application of 50 % Nitrogen, Phosphorus and 10 tonnes of farmyard manure (FYM) was applied along with last ploughing.

Planting time: Sweet sorghum can be grown during rainy season depending upon the availability of soil moisture/ irrigation sources and with suitable temperature regimes.

Rainy season crop (June –October 2012): Sowing of 18 genotypes was done at the end of june, 2013 immediately after the onset of monsoon.

Sowing was done on ridges with plant to plant distance of 10-15 cm on the row by hand dibbling.30 cm soil layer was fully charged with rainwater to ensure uniform germination.

Method of Planting: The crop was planted on flatbeds or on ridges or in furrows as similar to sugarcane.

Seed rate: 8 kg/ ha (or 3 kg /acre).

Soil type & depth: Deep black soil (Vertisol) or deep red loamy soil (Alfisol) with a soil depth of ≥ 1.0m deep. Planting on light shallow soils was avoided. The ideal pH range is 5.0-8.5.

Spacing: 60cm*15 cm; Row to row distance: 60 cm & plant to plant distance: 15 cm kept for raising crop.

Plant population About 10 plants per one m^2 maintained. A good plant population of brown midrib sorghum was maintained.

Thinning: 1) First thinning was done at about 15 days after planting (DAP) and two seedlings per hill at 15 cm apart retained. 2) At second (final) thinning at about 25-30 DAP retain single plant per hill. Thinning operation is very essential for uniform stand establishment and growth of plants. If this is not done very thin stalks of uneven size are produced due to competition for light, water and nutrients leading to crop lodging resulting in low yields. Lack of crop uniformity will also pose problem in deciding harvesting time too.

De-tillering: Side tillers that occur from the base of the plant were manually removed.

Intercultivation or hoeing: Inter-cultivation with blade harrow or cultivator once or twice between 20 and 35 days after sowing was carried out. This enabled the earthling up of the crop and prevented lodging.

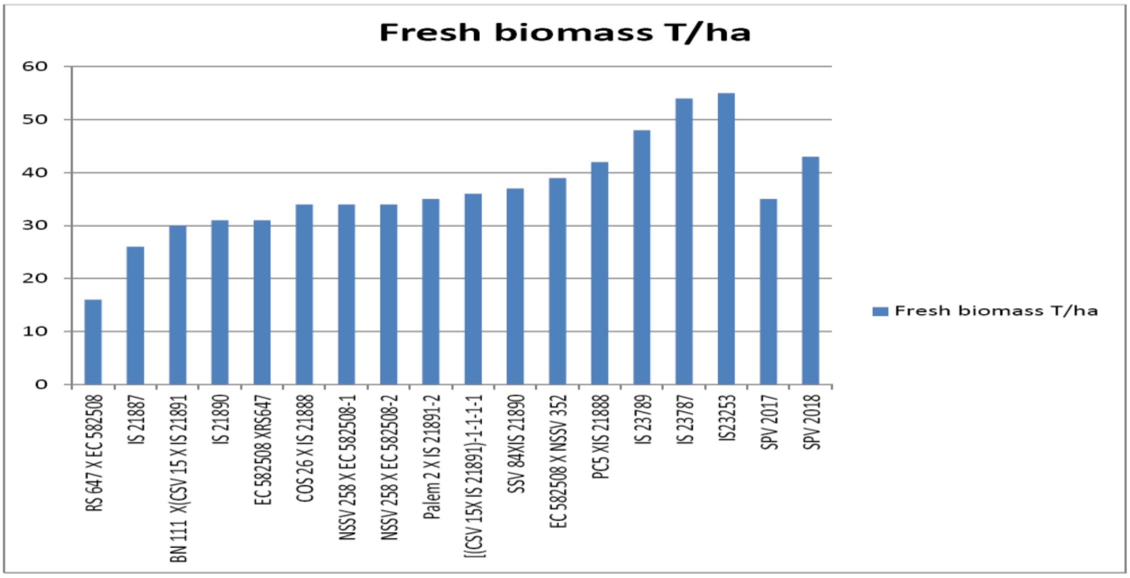
Brown midrib Sorghum genotypes evaluated for biofuel traits

Sr No	Genotypes	Plant ht	No of plants	DFF	DM	Stem girth	Fresh biomass(T/ha)	Dry biomass (T/ha)
1	IS23253	252	42	89	129	2.0	55	21
2	IS 23789	205	43	78	118	1.9	48	19
3	IS 21890	202	33	78	118	2.1	31	13
4	IS 23787	159	51	91	131	2.0	54	21
5	IS 21887	193	37	72	112	2.0	26	11
6	SPV 2017	242	52	90	130	1.8	35	15
7	SPV 2018	145	38	65	105	2.0	43	17
8	COS 26 X IS 21888	194	54	70	110	1.9	34	14
9	PC5 XIS 21888	196	59	73	113	1.8	42	17
10	Palem 2 X IS 21891-2	252	44	86	126	2.0	35	15
11	EC 582508 X NSSV 352	164	41	78	118	1.9	39	16
12	BN 111 X(CSV 15 X IS 21891	221	48	76	116	1.8	30	12
13	SSV 84XIS 21890	233	54	78	92	2.0	37	14
14	RS 647 X EC 582508	200	31	71	111	2.1	16	6

15	NSSV 258 X EC 582508-1	237	47	74	114	1.9	34	15
16	NSSV 258 X EC 582508-2	198	40	76	91	1.8	34	15
17	[(CSV 15X IS 21891)-1-1-1-1	249	53	79	119	1.8	36	14
18	EC 582508 XRS647	198	56	74	114	2.0	31	12
	Mean	208.7	45.7	77.66	114.8	1.9	36.6	14.8
	CD at 5%	40	15.74	3	3.5		12.5	5.3
	CV (%)	8.8	20.18	3.5	4.2	0.2	11.5	12.3
	F probability		0	0	0	0	0	0

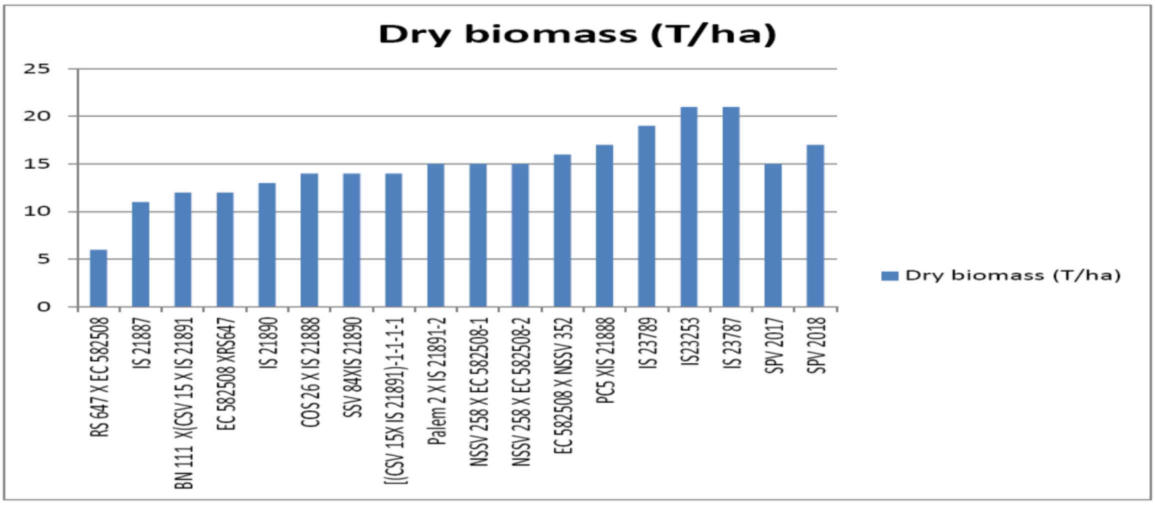
A brown midrib sorghum trial was conducted with 18 diverse bmr lines with derivatives in RBD in three replication. The fresh biomass ranged from 16-55 tons/ha and dry biomass ranged from 6 to 21 tons /ha.A total of three entries viz., IS 23253(55 ton/ha),IS 23787(54 tons/ha),IS 23789(48 tons/ha)SPV 2018(43 tons/ha)were superior compared to SPV 2017(35tons/ha).For dry biomass IS 23253 and IS 23787 were superior to SPV 2017 and SPV 2018.Plant height range from 145cm to252cm. Critical deviation and critical difference were measured. Data analysis was performed using INDOSTAT software. Graph shows variation in fresh and dry biomass for brown midrib parent and its derivatives.

Fresh biomass



Brown midrib parental lines and its derivatives were evaluated for fresh biomass and dry biomass. Variation in genotypes were observed for biomass. For fresh biomass IS 23787 (54T/ha) and IS 23253(55T/ha) were superior to check SPV 2017(35 T/ha) and SPV 2018 (43 T/ha).

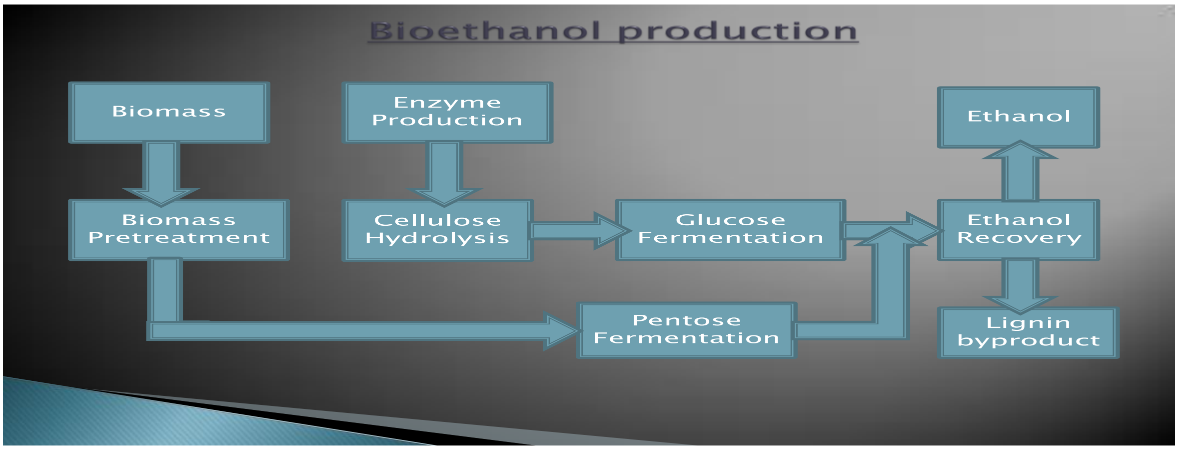
Dry biomass



Dry biomass is important trait for second generation cellulosic ethanol production. One parental line IS 23253(21 T/ha) and one brown midrib derivative IS 23787(21 T/ha) were recorded highest dry biomass yield .Significant differences were observed in dry biomass yields.

5. CONCLUSION

A brown midrib sorghum has high digestibility than other sorghum genotypes. The fresh biomass ranged from 16-55 tons/ha and dry biomass ranged from 6 to 21 ton/ha. Good biomass were recorded in few brown midrib genotypes ,IS 23253(55 ton/ha),IS 23787(54 tons/ha),IS 23789(48 tons/ha)SPV 2018(43 tons/ha)were superior compared to SPV 2017(35tons/ha).For dry biomass IS 23253 and IS 23787 were superior to SPV 2017 and SPV 2018.Sorghum cultivars with reduced lignin can pave a better way to increase second generation cellulosic ethanol production as compared with other crop residues and also improve process economics targeting higher conversion efficiency. Reduced lignin content will be highly beneficial for improving biomass conversion yield.



Diagrammatic Representation of biomass to bio-ethanol

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