Breeding Strategies for Sorghum as a Feedstock for First and Second Generation Technologies for Production of Bioenergy in Brazil

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Sweet sorghum in Brazil has the potential to be the "safrinha" or second crop after sugarcane to increase the processing period of the sugarcane distilleries (1st generation technology) by up to an additional 100 days per year. Sugarcane is processed from mid April to December for sugar and ethanol production, remaining idle for approximately four months each year. Sweet sorghum, planted at the beginning of the rainy season in October to December in idle areas of sugarcane renovation can be harvested from February to April and processed for ethanol production prior to the beginning of the harvest of sugarcane. The potential area of sugarcane renovation each year in Brazil is currently 1.8 million hectares which is expected to double during the next 20 years. Utilizing this area with sweet sorghum generates an initial demand for 14 thousand tons of seed which will be very difficult to meet with the use of varieties which require hand harvesting of plants nearly three meters tall. The solution is to develop high yielding, high quality sweet sorghum hybrids which can be mechanically harvested to meet the demand for seed. Currently, sweet sorghum hybrids in Brazil have yield potential of the released sweet sorghum varieties, but are inferior in quality. The lower quality is due the absence of sweet sorghum females and the use of low sugar juicy stem female lines. The development of maturity or "Period of Industrial Utilization" (PIU) curves is critical for industrial planning for delivery of the necessary quantity and quality of feedstock every day of the 30 to 100 day sweet sorghum milling period. The development and implementation of 2nd Generation technologies requires the development of both high yielding and high quality biomass production. Embrapa Maize and Sorghum is developing very high yielding (50 – 60 t ha^{-1} dry matter) high energy photosensitive hybrids as a feedstock for this technology. Strategies for decreasing or increasing lignin content, depending on the processing format are also being developed.

Breeding Goals for Sweet Sorghum

Sweet sorghum is being developed to be planted at the beginning of the rainy season in areas of Sugarcane renovation to increase the period of operation of large distilleries in Brazil by up to 100 days. Current revised breeding goals from our earlier program from 1975 - 1985 are presented below.

Sweet Sorghum Yield and Quality Breeding Goals

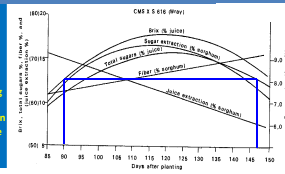
Trait	Minimum Target 1975	Minimum Target 2011
Minimum Biomass Yield	40 tha^{-1} (10 tha^{-1}/mo)	60 tha^{-1} (12-15 tha^{-1}/mo)
Minimum Brix* (245kg cm-1 for 60 seconds)	16 – 17º	18 -19º
Peak Brix*	21º	23º
Minimum ART Extraction* (Hyd. Press) (kg t^{-1} biomass)	80	100 – 120
Extraction Efficiency**	60-65 %	90-95 %
Minimum Total Sugar Content in Juice*	12.5 %	14 %
Minimum Alcohol Yield* **	40 l t^{-1} biomass	60 - 70 l t^{-1} biomass
90% Fermentation Efficiency**	90	95
Distillation Efficiency**	90	95
Industrial Efficiency**	81	90
Period of Industrial Utilization*	30 days	30days
Cultivar Type*	Variety	Hybrid

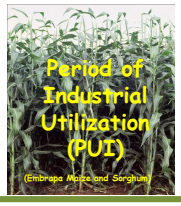
*Agronomic Parameters ** Industrial Parameters

Period of Industrial Utilization – PIU

The interaction of:
- Brix
- Total Sugars
- Fiber
- Juice Extraction
- Sugar Extraction

extracted from sorghum stalks during the maturity phase at 7 or 14 day intervals for the variety Wray grown in Brazil using a hydraulic press for juice extraction. (Embrapa Maize and Sorghum)

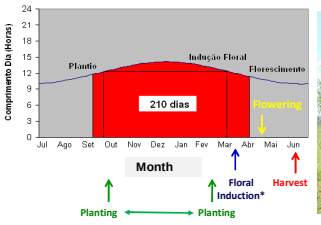




Breeding Goals for Biomass Sorghum

Energy or Biomass Sorghums are being developed for total biomass quantity as well as quality that depends on the type of 2nd generation technology to be used. Our strategy is to develop high yielding photosensitive hybrids using day length insensitive multiple stress female and photosensitive restorer lines with low and high levels of lignin.

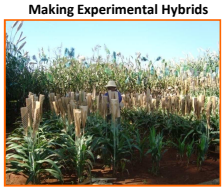
Maturity Cycle of Photosensitive Sorghum in Central Brazil – Up to 270 days



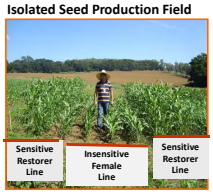
*Flowering is induced in photosensitive sorghum with daylength less than 12h 20m

Hybrid Seed Production

Making Experimental Hybrids



Isolated Seed Production Field



Small window or opportunity of simultaneous planting of male and female lines for simultaneous flowering for easy seed production (February 1012568146105 – March 1).

Hybrid	Fresh Weight t ha^{-1}	Fresh Weight t ha^{-1}	Dry Weight t ha^{-1}	Dry Weight t ha^{-1}
	Sete Lagoas	N. Porterinha	Sete Lagoas	N. Porterinha
200976005	109	120	25.8	57.9
200976006	99	113	30.8	49.7
200976007	100	99	34.1	44.0
200976029	87	96	25.4	43.2
200976030	122	122	40.6	53.1
200976031	74	90	21.4	43.8

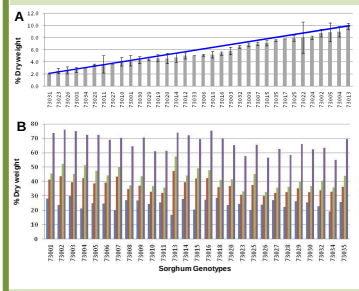
First year results of Experimental Hybrids at two locations at Embrapa Maize and Sorghum in Brazil, 2009/2010.

Breeding Strategies for Modifying Lignin Content

Our objective in developing Biomass Sorghums is to tailor the composition to the 2nd generation technology for transforming the biomass to electric energy or biofuels or bio-feedstock. Sorghum with a higher level of lignin is desirable for burning and with a lower level of lignin is desirable for hydrolysis of the cellulose and hemicellulos to sugars.

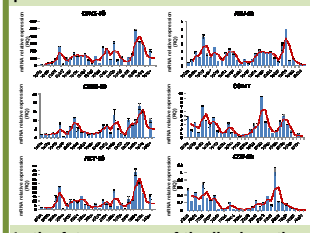
Analysis of lignin content variability in a genetically diverse Sorghum panel

A genetically diverse sorghum panel comprising 100 accessions was screened for lignin content and other cell wall components, using standard methods for compositional analysis and Klason lignin. Lignin content varied from 2 to 11% of total dry matter, and averaged 5.8%.



Composition analysis of a subset of the sorghum energy panel showing biochemical variability among genotypes. **A)** Lignin content varied from 2 to 10% of dry matter. **B)** Content for hemicellulose, cellulose, NDF and ADF are expressed as % of dry matter (Estimations for hemicellulose and cellulose were calculated as Hemicellulose= NDF – ADF; Cellulose= ADF - Lignin). Plants were grown under greenhouse conditions until grain reached maturity. Error bars are SEM of 3 biological replicates (Hemicel= hemicellulose).

In order to better understand lignin synthesis in sorghum, we have used Real-Time PCR to study the expression of sorghum homologs of key genes involved in the lignin biosynthesis pathway. Five of these genes, *C3H1, C3H2, HCT, COMT* and *F5H*, appear to be co-regulated as suggested by highly correlated expression levels in a subset of 35 accessions of the diversity panel.



mRNA relative expression (RQ) of sorghum lignin biosynthesis genes *C3H1, C3H2, HCT, F5H, COMT* and *CCR*. mRNA was extracted from superior internodes of 30-day old plants grown under greenhouse conditions. Gene expression was evaluated in a subset of the sorghum energy panel using Real Time PCR with SYBR Green detection. Error bars are SEM of 3 replicates.

In the future, genes of the lignin pathway will be evaluated and those showing differential expression among accessions that have contrasting lignin content will be validated by association analysis in order to identify superior alleles involved in lignin content and develop molecular markers for the breeding program.

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