Mendel
BIOTECHNOLOGY

seeding a sustainable future

International Conference on Sorghum for Biofuels

Houston, Texas
August 19-22, 2008

Bottlenecks in Feedstock Development: Can Biotechnology Address These?

Outline

- Supply chain/value chain structure
- Dedicated energy grass breeding goals & bottlenecks
- How do we address them?
  - Sorghum as genomics/biotech research tool for all dedicated energy grasses
  - How can we deploy biotechnology in sorghum commercial products?
  - Biotech traits – lessons from other crops

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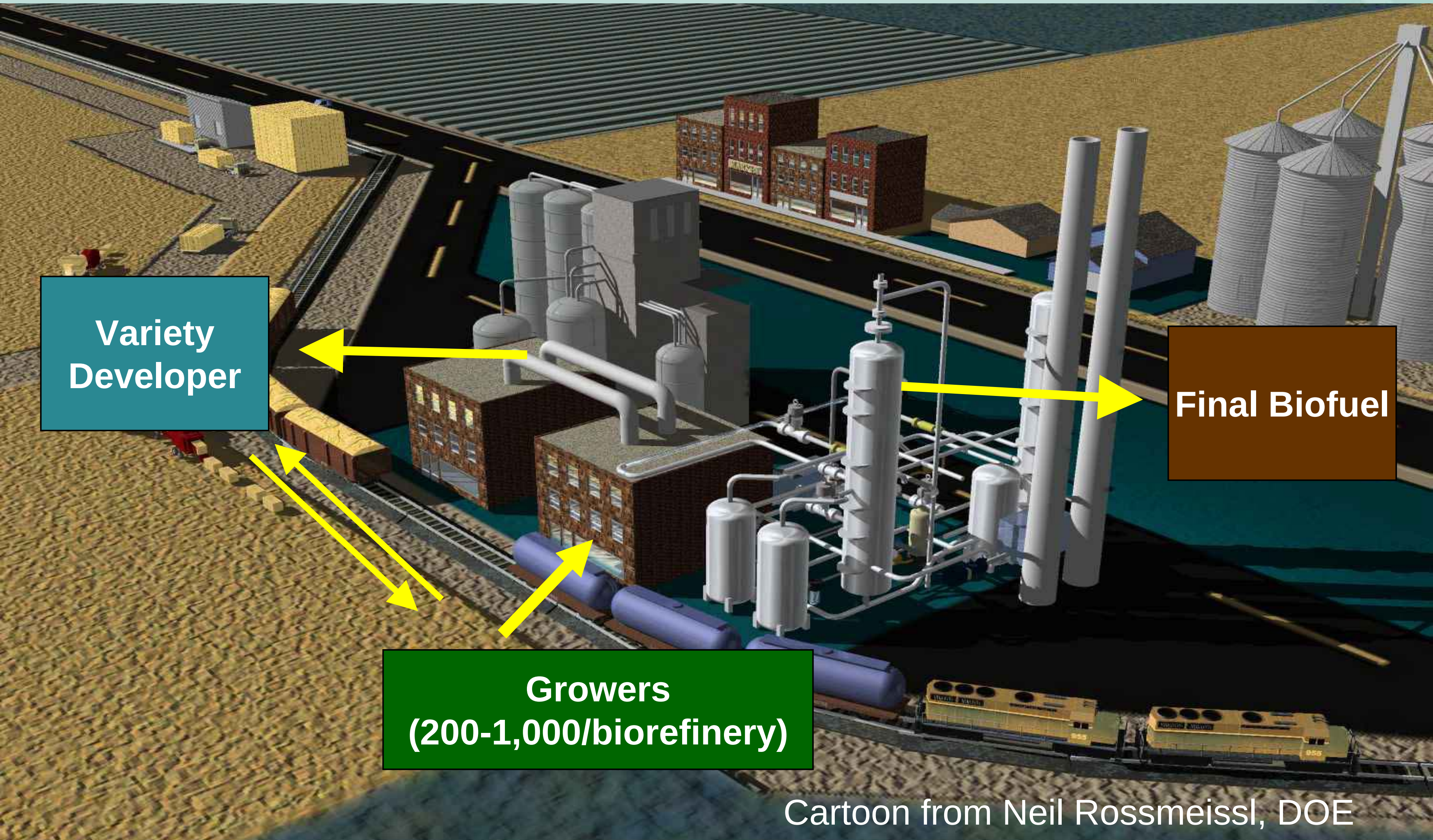
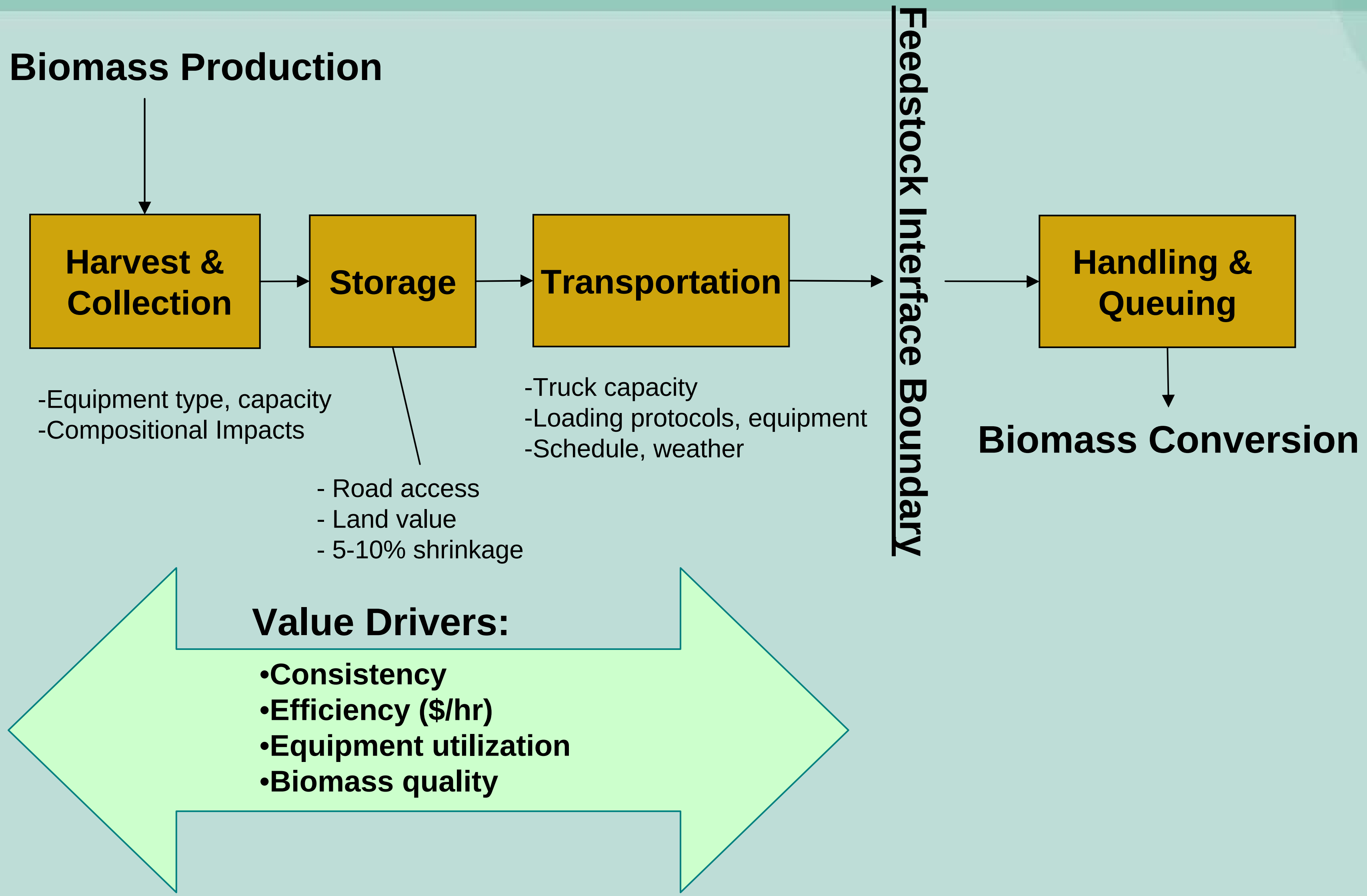
Biomass production & refinery operations will be locally integrated systems



Cartoon from Neil Rossmeissl, DOE

LC Biomass Supply Chain



Mendel Crop Portfolio

	Miscanthus	Energycane /Miscane	Sorghum
Short term	Vegetatively propagated varieties	Collaborate w/universities & companies	Existing forage breeding programs
Medium term	Seed propagated varieties	Miscanthus/ saccharum hybrids	"Biofuels"- type varieties
Long term	Seed propagated biotech varieties	Seed derived Miscane biotech varieties	Value-add varieties – biotech?

Biorefinery system establishment

Year	Perennial Grass Production	Annual Grass Need
1	0 dt	0 dt
2	700,000 dt	350,000 dt

This analysis reveals a critical role for an annual grass, particularly in the establishment period for a greenfield biorefinery.

“Sorghum safety valve”

Year	Perennial Grass Production	Sorghum Need
3	950,000 dt	50,000 dt
4	1,050,000 dt	None;
5	925,000 dt	75,000 dt
6	1,025,000 dt	25,000 dt
…..	……	……

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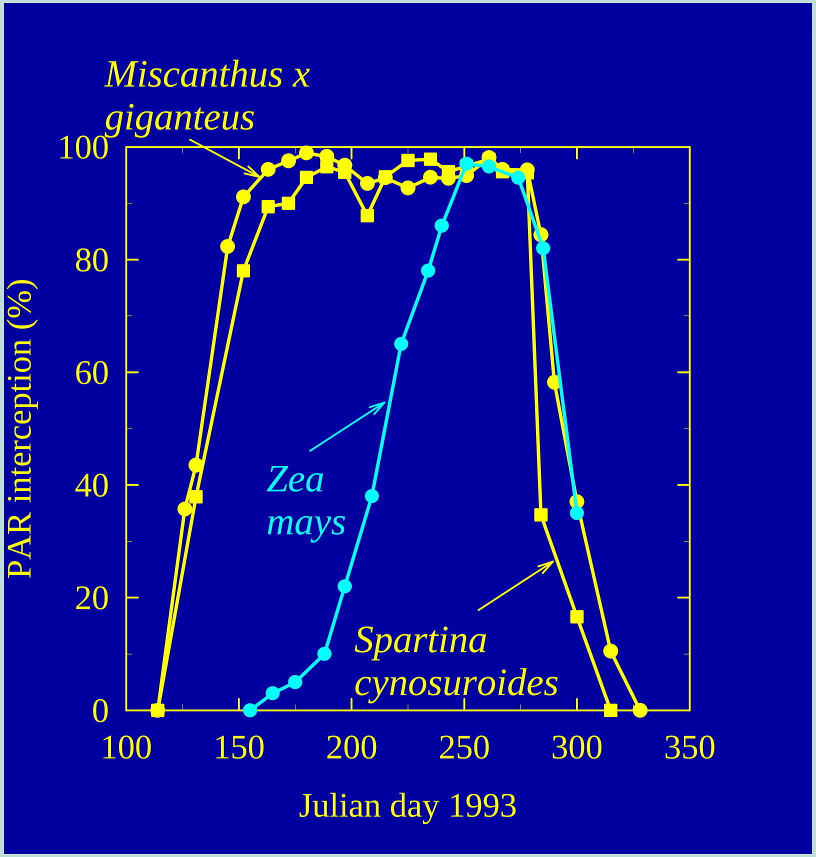
Advanced Biofuel Feedstock Breeding Goals – General Considerations

- **Higher biomass yield (>15 dry tons/acre/year)**
  - **~20% of 12 mi radius = 100M gal/year**
- **Reduced input (fertilizer, water, tillage, pesticides)**
- **Improved sustainability**
- **Stable quality from year to year**

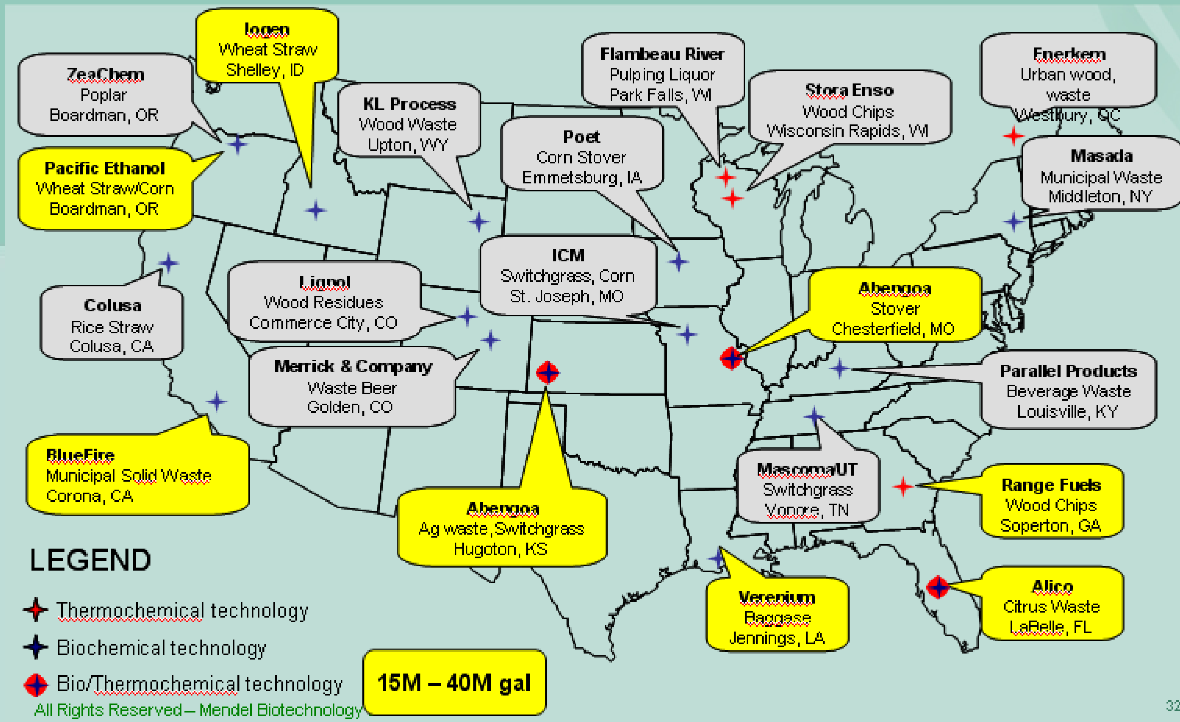


Finding the customer – to define more specialized development goals

- Increased energy yield/acre

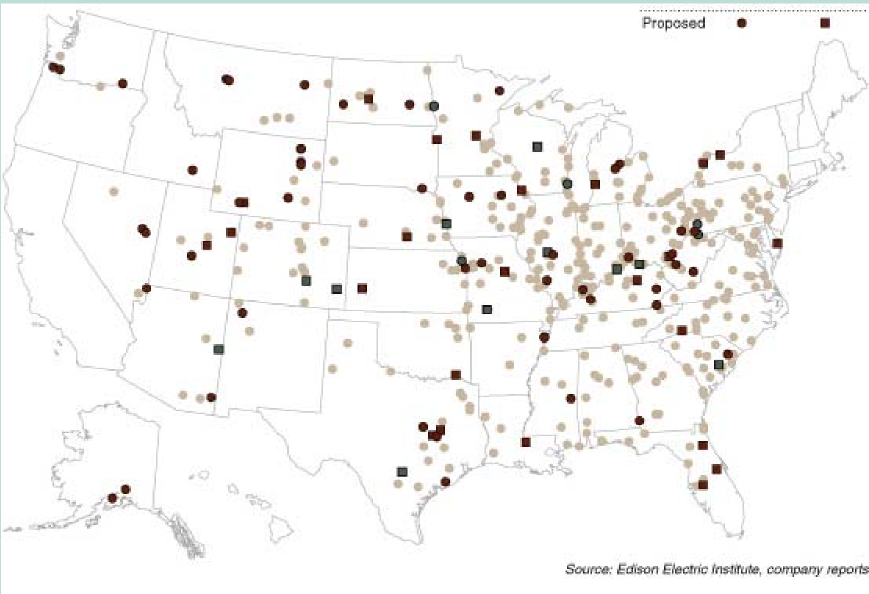
- What are the end markets?
  - Biorefineries
    - Thermochemical or Biochemical conversion
  - Power generation facilities
    (e.g., coal-firing)

- Breeding targets may be very different
  - Increased/decreased lignin content
  - Importance of water content, nutrient content



How variable are the customer needs? For example:

- ~ Lower lignin and readily “resolved” lignin for biorefinery applications
- ~ Increased lignin for higher energy density per acre for co-firing



Mendel initial Miscanthus products

- Clonal varieties
  - ~ Amuri, Nagara
- Seeded varieties
  - ~ Differing maturity groups
  - ~ Not yet named; being trialed across U.S. & Canada

Sorghum improvement

- Mendel has entered into a long-term collaboration with MMR Genetics to develop new "biofuels" varieties of sorghum
  - ~ Based largely on a range of forage types
- Sorghum is perhaps the most tractable genetic system amongst the 4 major genera under consideration
  - ~ A sorghum experimental model system would be advanageous
    - ~ Short flowering time, low light-requiring sorghum model to support variety improvement across andropogoneae
    - ~ This would support identification of biotech improvements that could be deployed in sorghum and related species

Major bottlenecks for biiotech-generated sorghum improvement

- Sorghum halapense
  - ~ Fear of exacerbating problems with this globally significant noxious weed
  - ~ No biotech applications for grain sorghum

- Sorghum production systems that satisfy sustainability principles for biofuels
- Trait-QTL associations

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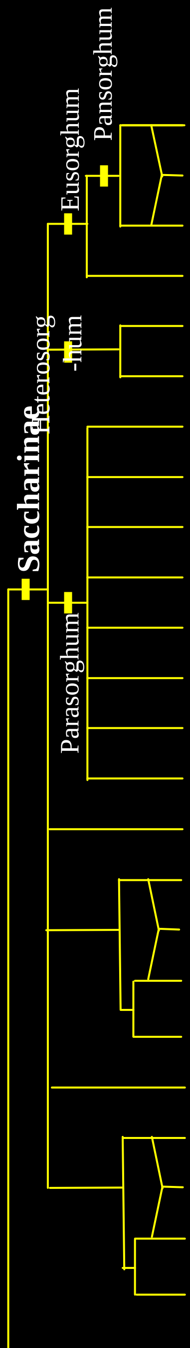
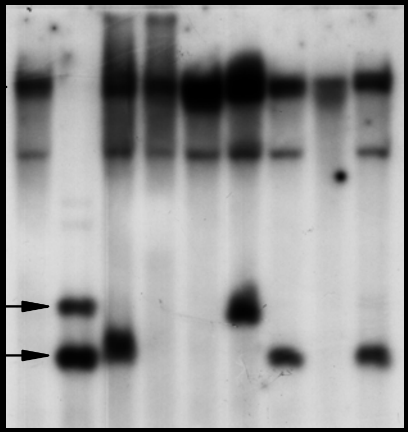
Sorghum is closely-related to sugarcane and Miscanthus



Species	2n=	Mbp/1C=
Sorghum bicolor	20	736
Sorghum halepense	40	?
Sorghum propinquum	20	690
Sorghum nitidum	20	4307
Sorghum macrospermum	40	1014
Sorghum laxiflorum	40	1220
Sorghum angustum	10	1813
Sorghum intrans	10	1117
Sorghum leiocladum	10	2254
Sorghum plumosum	30	3748
Sorghum purpureo-sericeum	10	2048
Sorghum timorense	10	622
Sorghum trichocladum	?	?
Sorghum versicolor	10, 20	1592, 3268
Cleistachne		
Miscanthus sinensis	38	2824-3786
Miscanthus x. giganteus	57	3916-4576
Miscanthus sacchariflorus	76	2234-2867
M. sacchariflorus 'Robustus'	38	
Microstegium nudum		
Saccharum spontaneum	36-128	
Saccharum cultivars	100-130	4183
Saccharum officinarum	70-140	
Saccharum robustum	60-170	
Zea mays	20	2300

Intergeneric hybrids retain both sugarcane (15.7kb) and sorghum alleles (3.5, 2.8 kb)



Courtesy of Andrew Paterson

Dedicated energy grasses genomes will be aligned to Sorghum map

- Within 3-4 years expect large amounts of genome sequence from 4 closely related Andropogoneae species:
  - ~ Zea, Saccharum, Sorghum, Miscanthus

- Sorghum and Zea likely anchors for increasingly informative Andropogoneae integrated genome-wide views

Deploying biotech traits in sorghum

- Gene flow from domesticated sorghum into *S. halapense* may – or may not – create an increased weediness problem
  - Premise: such gene flow is not likely to be acceptable from a public perspective, or a regulatory agency perspective

- While it may not be possible to commercialize biotech traits in grain sorghum, it could be more readily done in "biofuels" sorghum
  - Multiple approaches to sterility:
    - Triploidy, via crosses between diploid and tetraploid species
    - "Operational" sterility via photoperiodic loci that delay flowering past the end of the growing season
    - Biotech sterility via ablation of pollen production
  - Two systems may be necessary – "belts and suspenders"

Traits for corn – a recent Mendel example of enhanced drought tolerance

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PNAS PNAS

Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres

Donald E. Nelson*†, Peter P. Repetti‡, Tom R. Adams*, Robert A. Creelman‡, Jingrui Wu*, David C. Warner
Don C. Anstrom*, Robert J. Bensen*, Paolo P. Castiglioni*, Meghan G. Donnarummo*, Brendan S. Hinchey
Roderick W. Kumimoto‡, Don R. Maszle‡, Roger D. Canales*‡§, Katherine A. Krolikowski‡, Stanton B. Dots
Neal Gutterson‡, Oliver J. Ratcliffe‡, and Jacqueline E. Heard*‡§

*Monsanto Company, 62 Maritime Drive, Mystic, CT 06355; and ‡Mendel Biotechnology, Inc., 21375 Cabot Boulevard, Hayward, CA 9454

Communicated by Maarten J. Chrispeels, University of California at San Diego, La Jolla, CA, August 29, 2007 (received for review Decemb

Commercially improved crop performance under drought conditions has been challenging because of the complexity of the trait and the multitude of factors that influence yield. Here we report the resu
transcri

proteins that belong to the AP2/ethylene-resp binding protein family (11, 12). These factors enha the expression of genes with a CBF/DRE box in major stress tolerance pathway, in ABA) biosynthesis/response pathw

Fig. 1. Constitutive expression of *AtNF-YB1* confers drought tolerance in *Arabidopsis*. Representative pots of transgenic plants (*Left*) and controls (*Right*) are shown at the end of a dry-down period (*Upper*) and at 5 days after rewatering (*Lower*).

Fig. 5. Transgenic maize plants in greenhouse and field have visually observable improved drought tolerance. In both photographs, controls are in the left flat or row, and transgenics expressing ZmNF-YB2 are in the right flat or row.

…and a Mendel biotech trait for yield enhancement

Increased Corn Yield (bu/a) (Courtesy of Monsanto Co.)

	Broad Acre Yield		High density Yield
Event	Year 1	Year 2	
1	3.9	-2.22	-5.3
2	0.51	-1.86	2.8
3	2.33	5.41	7.81
4	5.21	2.61	8.21
5	1.13	-3.59	5.1

Percent change shown as compared to negative segregant.

Yield efficacy demonstrated at normal and high planting density for 2 events

Summary & perspectives

- Sorghum can be an excellent tool for both conventional genetics and biotech trait development for other dedicated energy crops
  - A sorghum model should be given priority
- Sorghum is probably the most rapidly improvable of the major dedicated energy crops
  - But biotech trait deployment, critical for long-term improvement, will likely require systems that minimize gene flow

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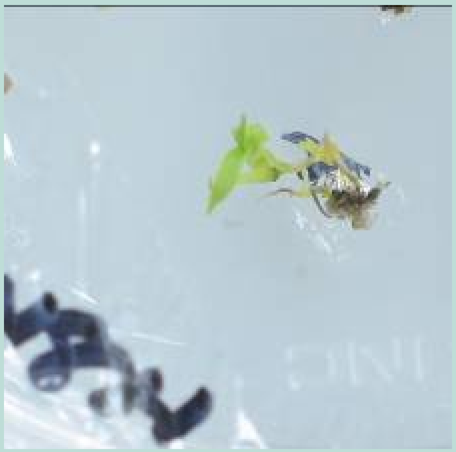
Thanks!

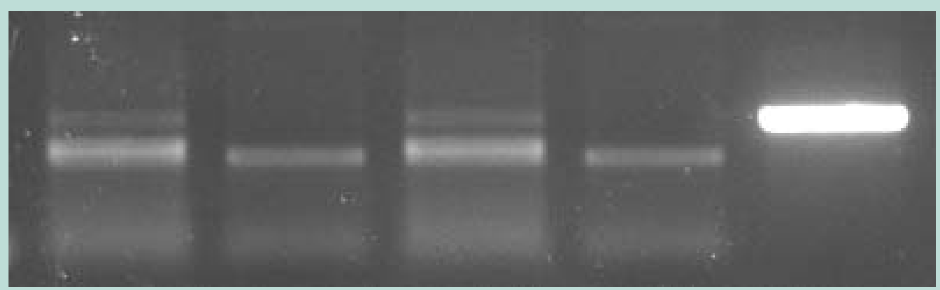
Mendel has developed Miscanthus transformation capability



transgenic *Miscanthus Sinensis*

Gus positive

Contains GUS DNA



Transgenic leaf #1	Control leaf #1	Transgenic leaf #2	Control leaf #2	plasmid