Genetics, Genomics, and Fundamental Aspects of Plant Breeding - Switchgrass

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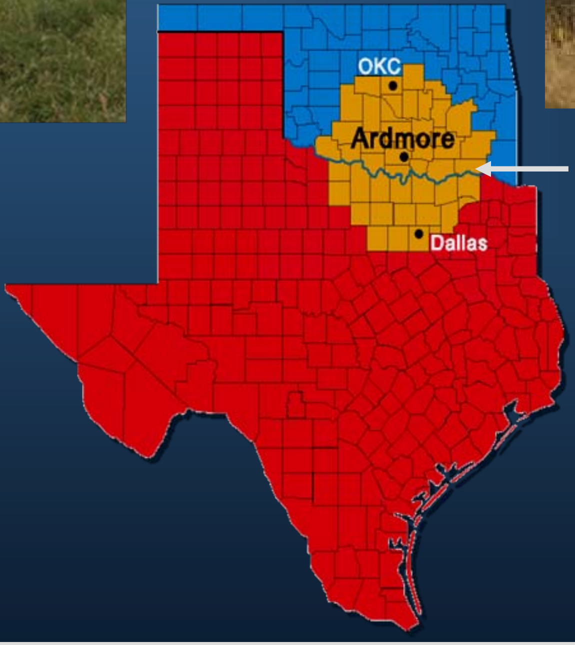
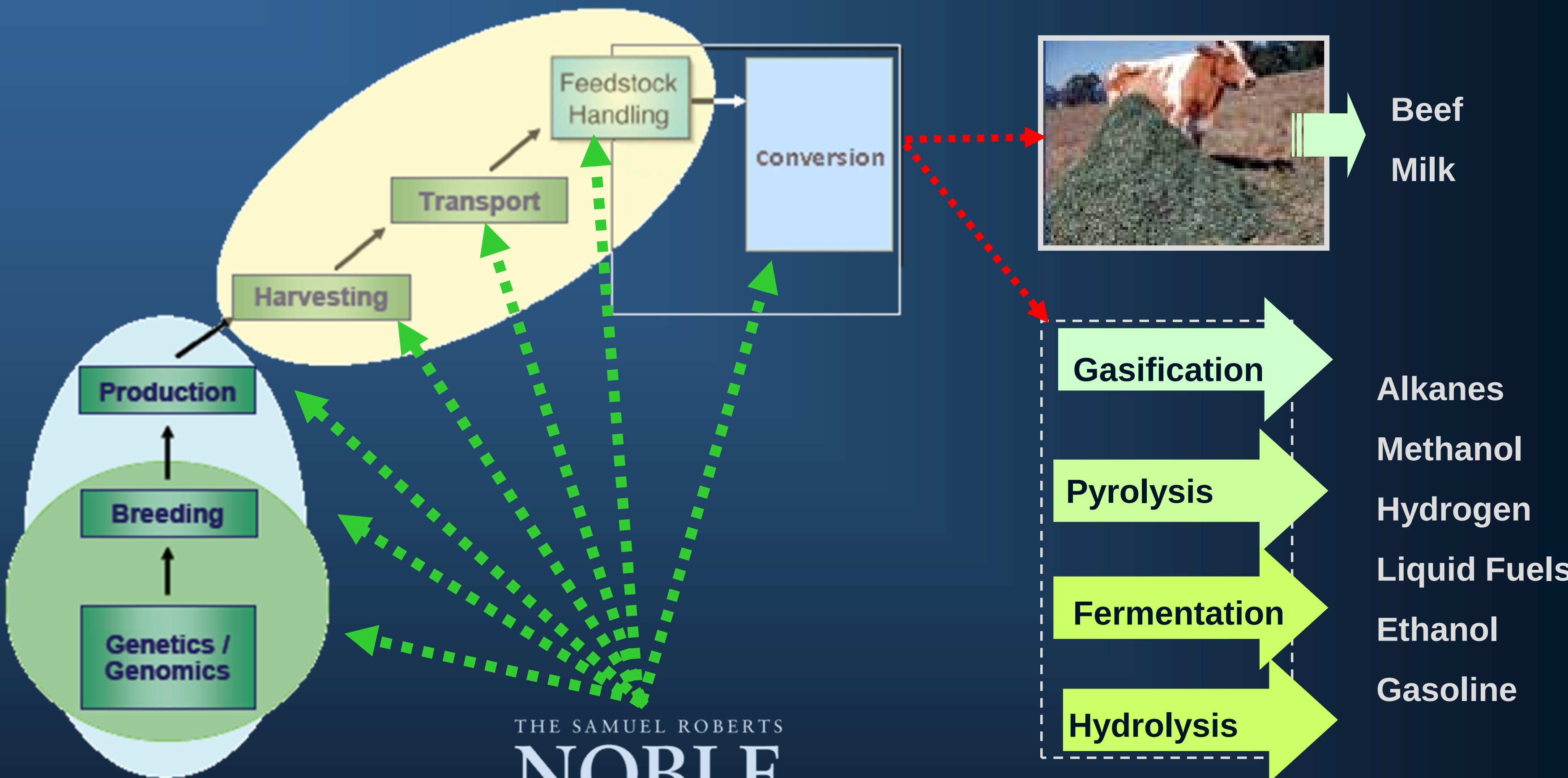
The Samuel Roberts Noble Foundation
Ardmore, Oklahoma

So. Great Plains Region & the NF service area has a long history of grass based agriculture and a strong oil and gas industry



If the NF service area (in orange) was a State in the USA, it would have the 10th largest cattle herd.

So for NF, the question is it biofuels or forage/livestock?



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Answer: It better have use for both areas, especially in the early days

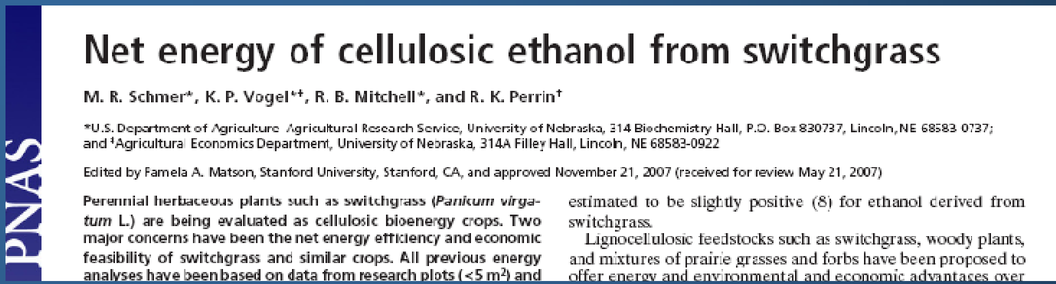
Stocker Cattle Performance

Utilizing Switchgrass after "Graze-Out" Small Grains Shows Great Promise

Stocking Rate	On wgt, lb/hd	Int. stocking rate, lb/ac	Days on pasture	ADG	Gain/hd (lbs)
3 hd/ac	771	2315	21	3.37	71
2 hd/ac	782	1563	29	2.63	76
1 hd/ac	786	786	70	2.16	151

From Billy Cook, NF AG Division, unpublished

How good is switchgrass as a biofuel crop?


PNAS

Net energy of cellulosic ethanol from switchgrass

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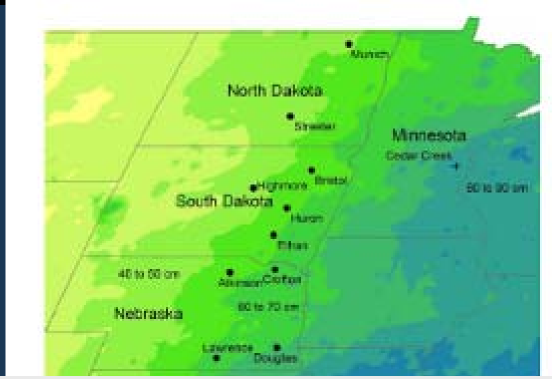
Edited by Pamela A. Matson, Stanford University, Stanford, CA, and approved November 21, 2007 (received for review May 21, 2007)

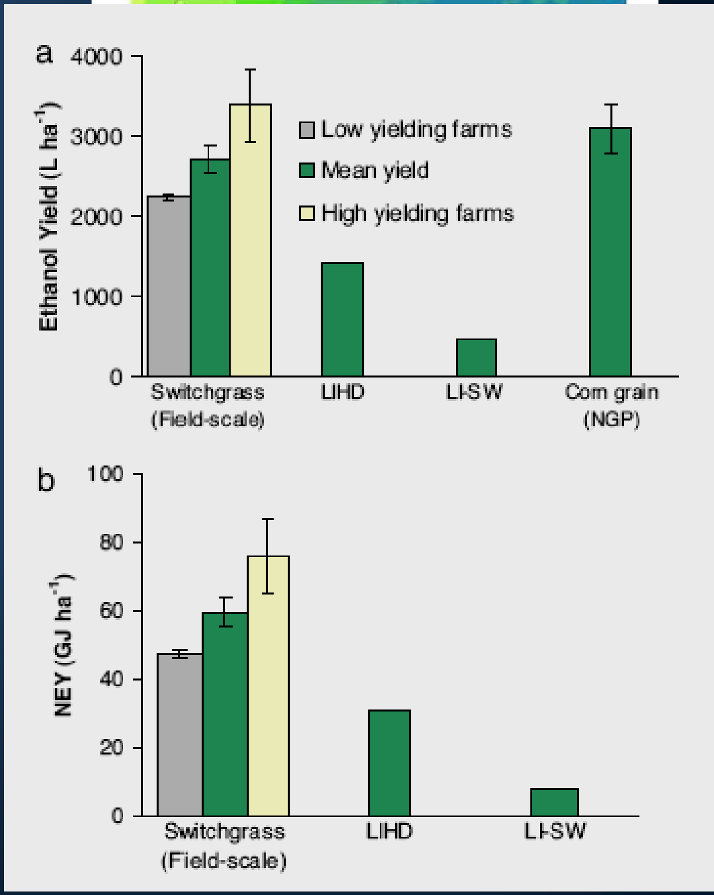
Perennial herbaceous plants such as switchgrass (*Panicum virgatum* L.) are being evaluated as cellulosic bioenergy crops. Two major concerns have been the net energy efficiency and economic feasibility of switchgrass and similar crops. All previous energy analyses have been based on data from research plots (<5 m²) and

estimated to be slightly positive (8) for ethanol derived from switchgrass.

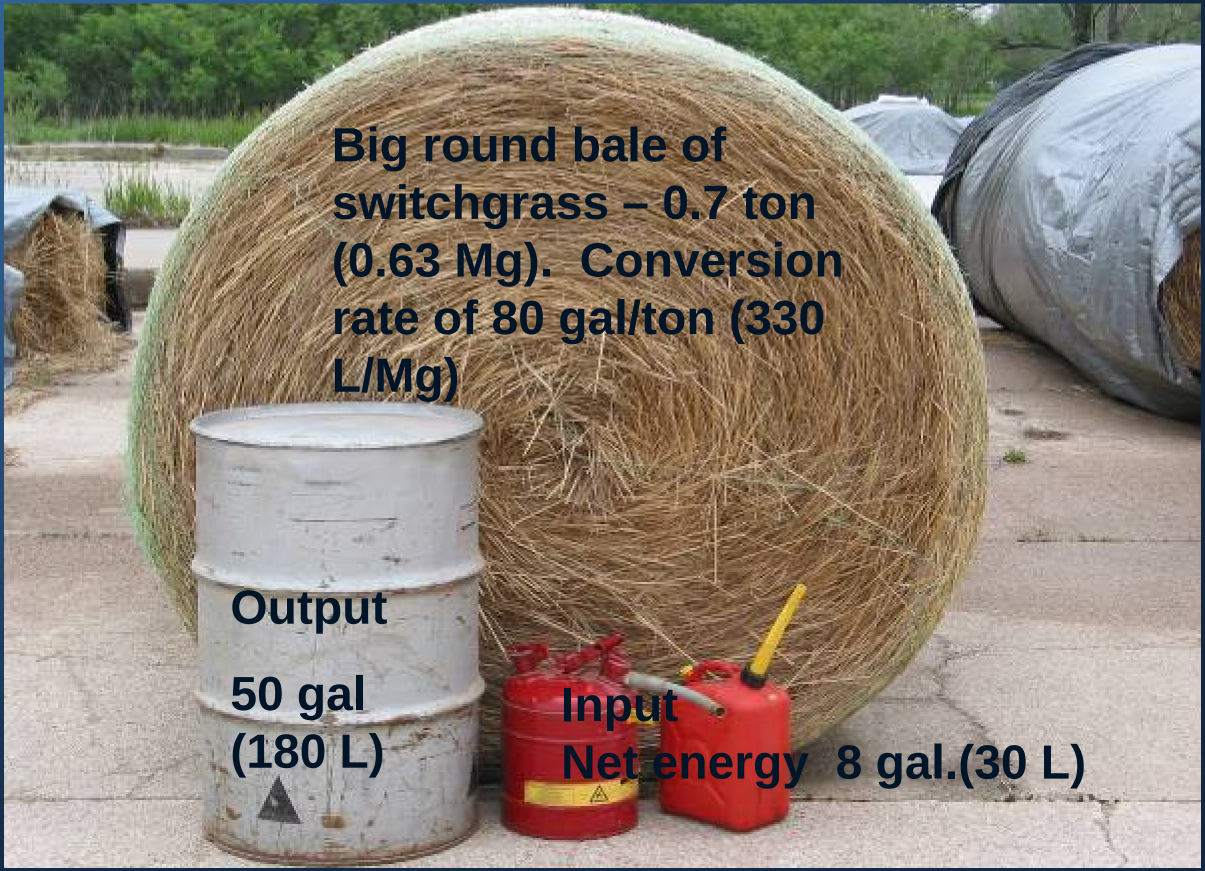
Lignocellulosic feedstocks such as switchgrass, woody plants, and mixtures of prairie grasses and forbs have been proposed to offer energy and environmental and economic advantages over



- **Seminal work published in Proceedings of the National Academy of Sciences (105:464-469).**
- Done **on-farm** (10 sites in Northern Great Plains)
- **Marginal crop land** (e.g. non-corn)
- High yield sites (5.5 tons/acre) produced **ethanol yields comparable to irrigated corn and 93% more than human-made prairies**
- Switchgrass produced **540% more energy than used**
- **New and improved varieties** and management practices should further increase performance since this study was first established in 2000.



Ethanol from switchgrass: Input - output illustration



Based on Schmer et al., 2008. PNAS105: 464-469.
(Slide courtesy of Ken Vogel, USDA-ARS)

What is Switchgrass? What does it look like?

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From Bouton, Current Opinion in Genetics & Development 2007, 17:553–558

Switchgrass Cytotypes: Uplands vs. Lowlands

- **Lowlands are tall, coarse** plants with exceptional biomass yields, and generally **found in wet areas with mild winter temperatures.**
- **Lowlands** are predominately **tetraploids (2n=4x=36).**
- **Uplands are shorter in stature** than lowlands, **lower in biomass**, mainly collected in **drier, colder zones**, and are **mainly octoploids (2n=8x=72) with some tetraploids.**
- **No natural crossing occurs across the ploidy groups** therefore upland and lowland types are reproductively isolated for the most part.

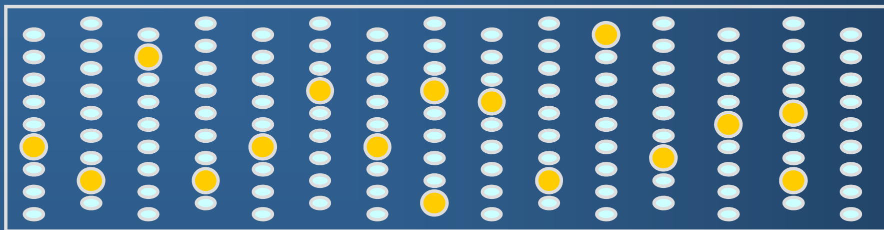
From Bouton, Current Opinion in Genetics & Development 2007, 17:553–558

Breeding and Cultivar Development

- **Initial cultivar development** centered on **accession or ecotype collections**, screening these in field trials for performance and geographic adaptation, and then **directly increasing seed and releasing** the best accession population as a new cultivar.
- Cultivars such as **Alamo, Blackwell, Kanlow, Cave-in-Rock, and Nebraska 28** were released in this manner.
- However, since switchgrass is a cross-pollinated perennial, **recent breeding methodologies include among and within family selection methods** with the eventual **identification of superior genotypes** to be used as **parents for synthetic cultivars.**
- Modern cultivars produced in this manner now being sold include **Trailblazer, Shawnee, and Pathfinder.**

From Bouton, Current Opinion in Genetics & Development 2007, 17:553–558

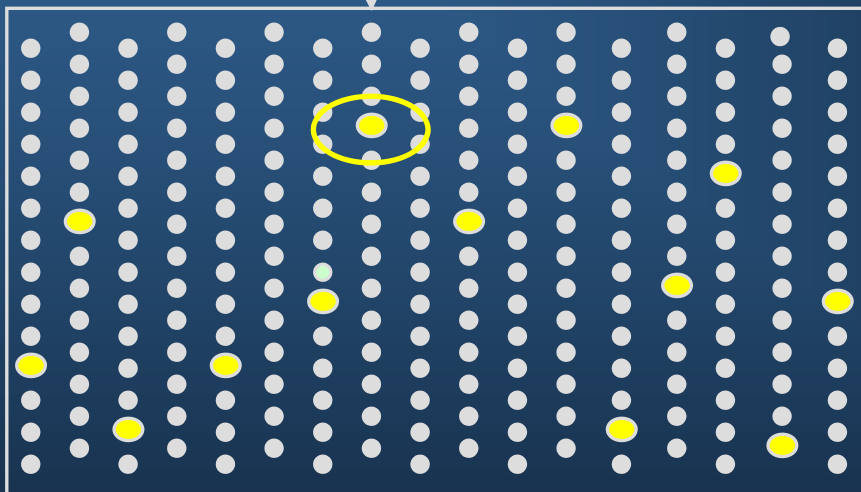
Development of Synthetic Cultivars
Half-sib Among & Within Family Selection Method



Initial nursery

-Best Breeding Germplasms

Phenotypic selection best genotypes (biomass, seed)



Half-sib families planted in **honeycomb design** (**also see Fasoulas and Fasoula, 1995**), at multi-locations, with multi-genotypes per family at each location.

Synthetics population will be developed from
-Parent genotypes of the superior families
-Superior genotypes select from the superior families

This also serves as the base population for the subsequent cycles

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From Bouton, Current Opinion in Genetics & Development 2007, 17:553–558

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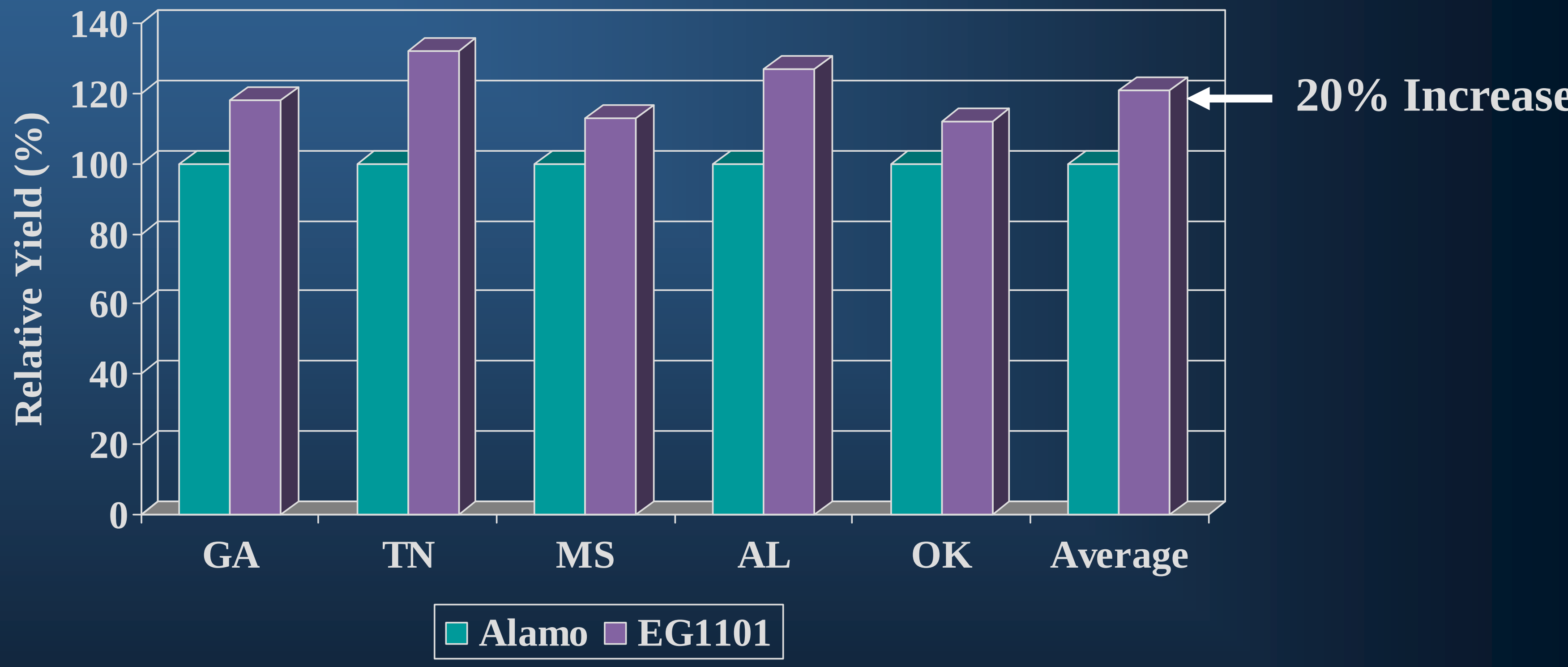
Example of Progress Via the Half-Sib, Among and Within Family Selection Method:

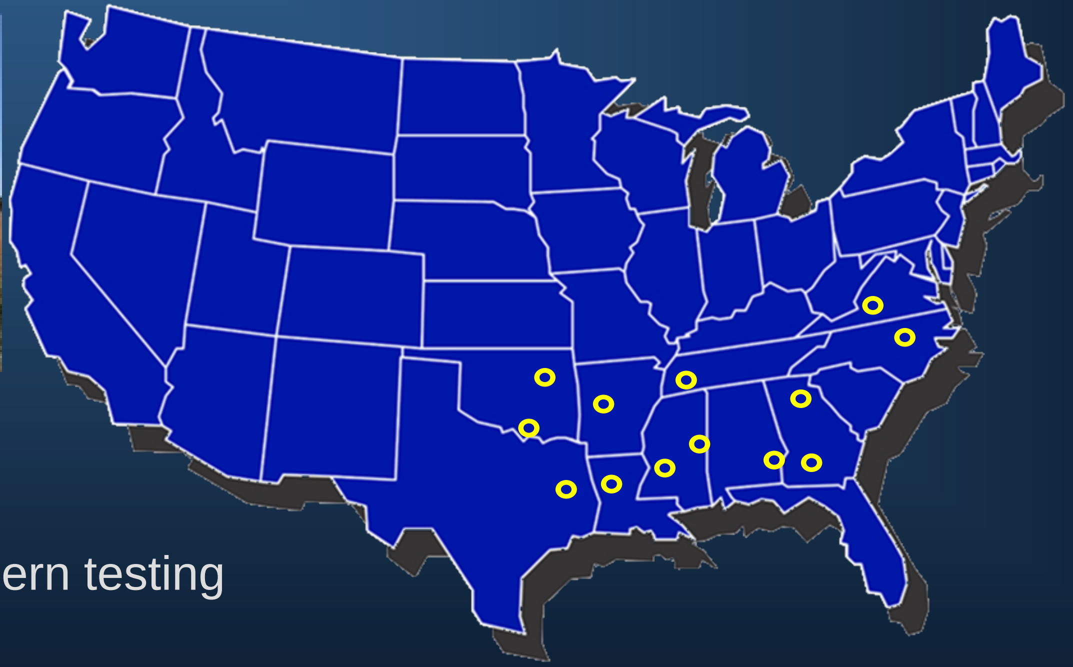
Dry Matter Yield Performance of EG1101 (tested as NF/GA993) Switchgrass Variety Relative to the 'Alamo'



Commercial seed should be available in 2009-10 through Blade™

NF Switchgrass Research Projects

- **Commercialization and testing of EG1101 and EG1102**
- **Establish better management protocols for growers**
- **Development of new cultivars including F1 hybrids**
- **Develop & apply biotech tools for breeding new cultivars**



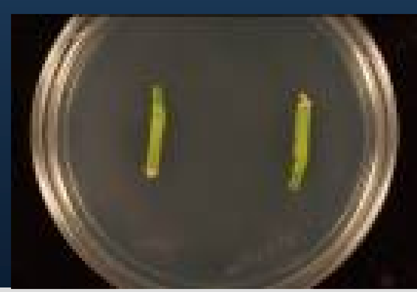
Developing F1 Hybrids Cultivars?

Findings from Vogel (1998) & Martinez-Reyna and Vogel (2008):

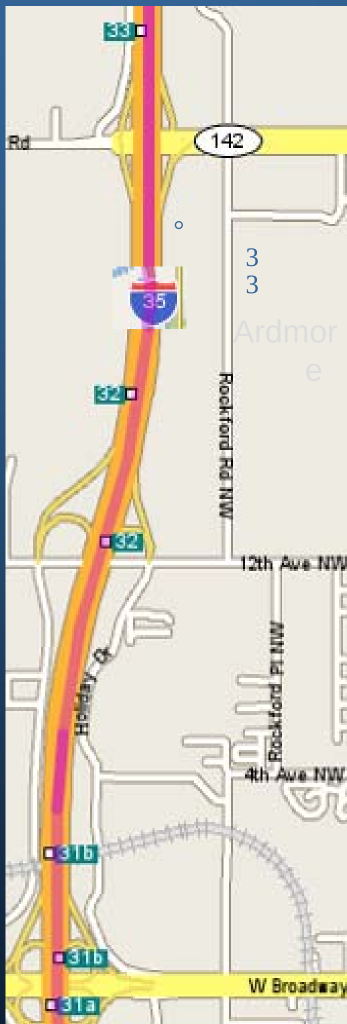
- Feasible to develop hybrid switchgrass cultivars by using its self-incompatibility system.
- Heterosis exists in switchgrass for several agronomic traits including biomass yield.

Findings from Conger (1999):

- Thousands of switchgrass plantlets can be produced from a single parent plant through tissue culture techniques and brought to field-ready status in a period of three months.
- Useful to develop isolated breeding blocks of two superior parent plants for developing F1 hybrids.

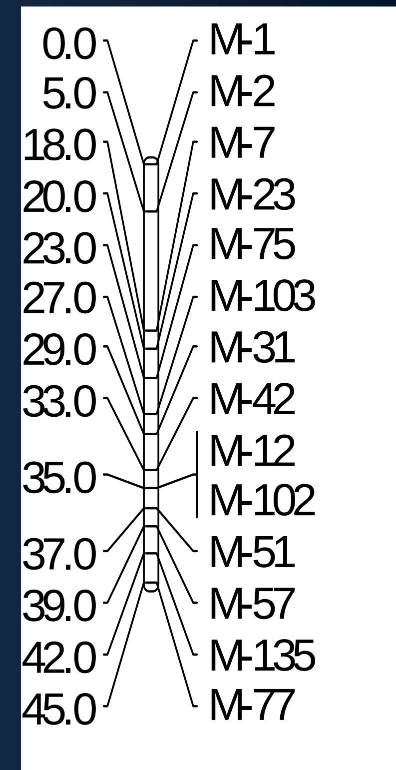


Why use biotech and genomic tools such as molecular markers and gene mapping?



Analagous to Road Maps

Chromosome 1

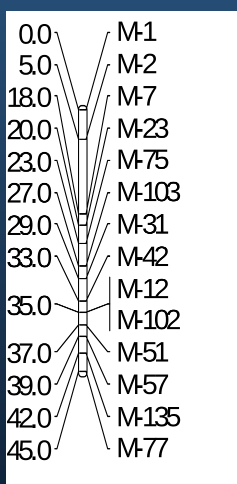


- Chromosomes are like the highways.
- The molecular markers on the chromosome are like the mile markers on a road map.
- Easier to find a stalled car on the road if you report the mile marker it is near.
- It is the same for a gene. It is easier to find the gene if it is associated (linked) with a marker that you can find and easily manipulate.

Molecular markers in switchgrass

- Need to develop molecular markers specific for switchgrass
- Available resources

Chromosome 1

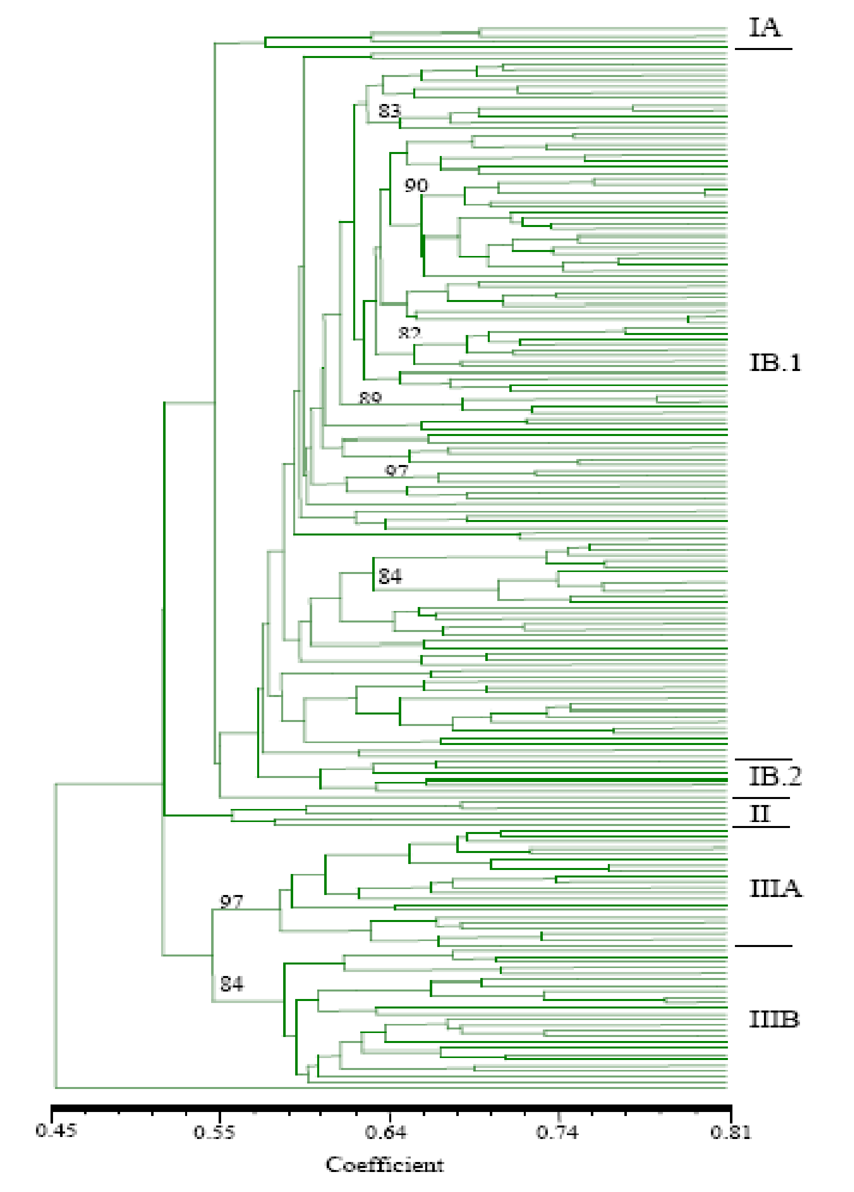


Conserved Grass (CNL) EST-SSRs

Switchgrass EST-SSRs → Private / Public

Genomic SSRs are in development at NF

Example: Assessing diversity in the USDA GRIN switchgrass collection via molecular markers



- ☞Three major clusters I, II,&III
- ☞ Cluster 1– upland
- ☞Lowlands differentiates into groups IIIA&IIIB
- ☞ Few instances of lowland accessions clustered with upland accessions

From Narasimhamoorthy.B, Saha MC, Swaller T and Bouton J. 2008. *Bio-energy Research* (Online first)

Example: Genetic mapping of important traits for marker assisted selection

Two sets of parents, AP13 and VS16, 251 F1 progeny, and a 'Alamo' check are planted in field using the 'R-256 Honey Comb' design

AP13
4X, Lowland

VS16
4X, Upland

Also see Missaoui , Paterson, Bouton. 2005. *Theor. Appl. Genet.* 110:1372-1386.

Variation in traits with potential for genetic mapping

- Spring re-growth
- Heading-Julian days
- Dormancy
- Plant color
- SPAD readings
- Steminess /plant type
- Plant height
- Tiller number
- Seedhead type
- Disease resistance
- Maturity/senescence
- Biomass
- Seed yield
- Composition


Early vs late senescence


Bluish stem and leaf


Variability in inflorescence

“Phenotyping” the Mapping Population

Source	Re-growth (J. day)	Leaf length (cm)	Leaf width (cm)	Leaf color (1-5)	SPAD reading	Rust score (1-9)
AP13 mean	85	40.8	1.26	4.8	44.5	3
VS16 mean	93	26.1	0.76	1.1	41.0	7
F1s mean	89	30.6	0.98	2.5	46.8	5
F1s Range	71-94	25-41	0.7-1.4	1.0-4.8	36-54	3 -7.4
LSD	6.8	4.01	0.20	0.90	9.41	0.98

Severe rust infection

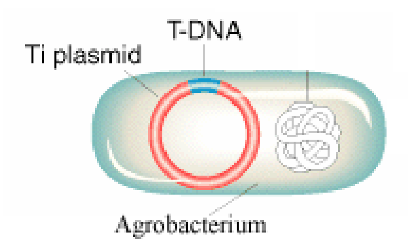
Puccinia emaculata

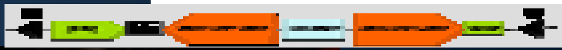
Susceptible

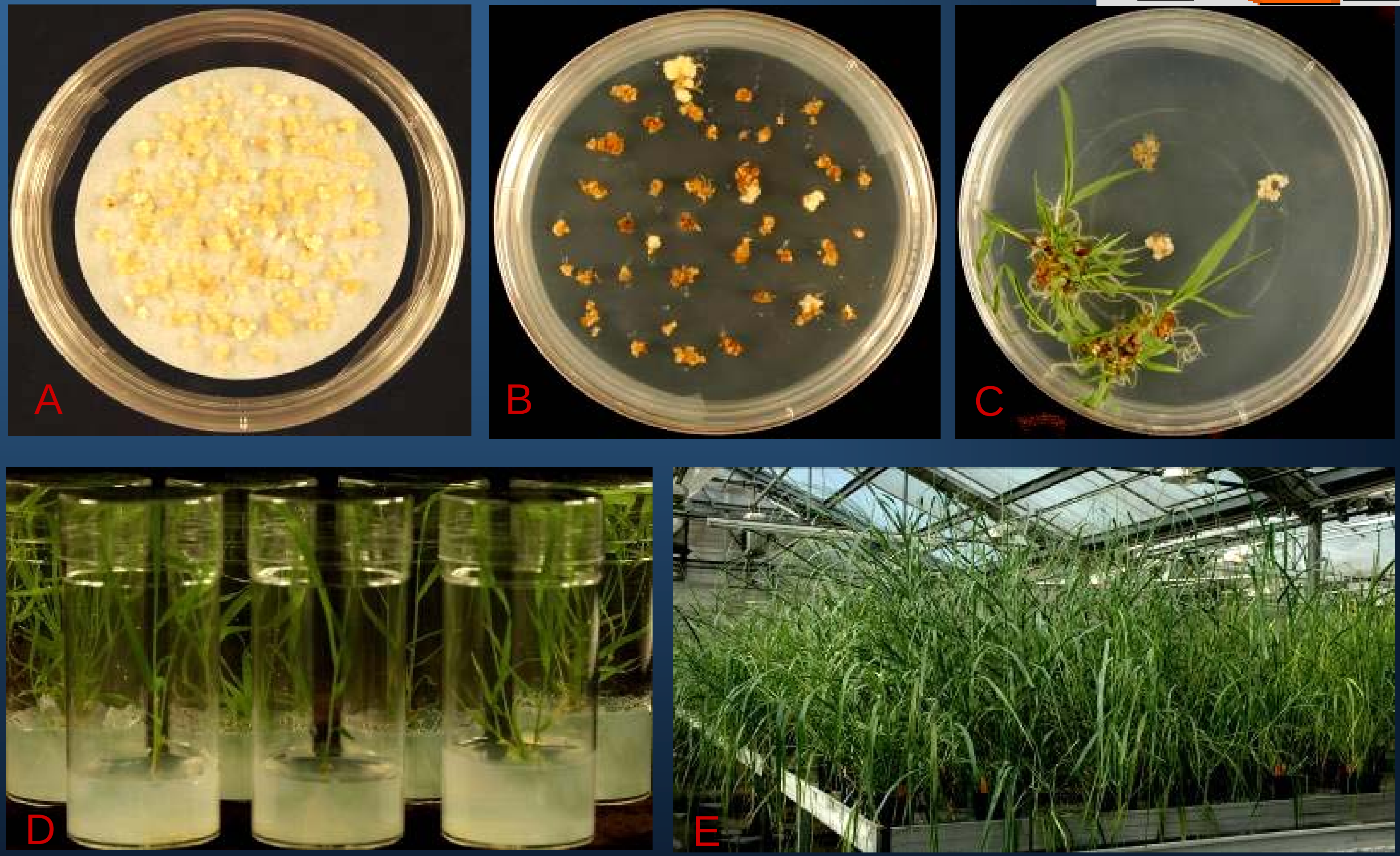
Resistant

Transgenics: The Ultimate Biotech Tool

Transgenic switchgrass obtained by *Agrobacterium*-mediated transformation







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Potential Biotech Transgenes from the Noble and the Ceres Gene Discovery Pipelines

High biomass via increased growth rate, higher photosynthetic efficiency, photoperiod insensitivity

Disease and pest resistant

Improved processing; **low lignin** and better sugar yield

Roots, **nutrient transport, salt tolerance**

Confinement: no pollen, no seeds

Cold germination, cold growth dense planting

Perennial, **optimized architecture**, shade tolerant

Deep roots: **drought tolerance**, nutrient uptake, carbon sequestration

Summary: Achieving the Biomass & Ethanol Yield Goals for Switchgrass via Breeding and Genomics