2007 Symposium on Biomass Feedstocks for Energy Production in Illinois

Miscanthus Breeding and Improvement

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Rationale for *Miscanthus* Genetic Improvement

- Global market for biofuels will grow to an estimated $10 billion by 2012 (Shell Oil).
- Bioenergy crops needed as alternative to reliance on international sources of oil and coal.
- *Miscanthus x giganteus* yields 2-3 times more biomass than switchgrass in the Midwest (Heaton et al. 2004).
- No previous significant efforts to develop genotypes adapted to Midwestern growing conditions.

Approaches to *Miscanthus* Genetic Improvement

- Conventional breeding to generate new genotypes of *Miscanthus x giganteus*.
- Plant regeneration, somoclonal variation, and chromosome doubling.
- *Miscanthus x giganteus* transformation to introduce beneficial genes into existing genotypes.

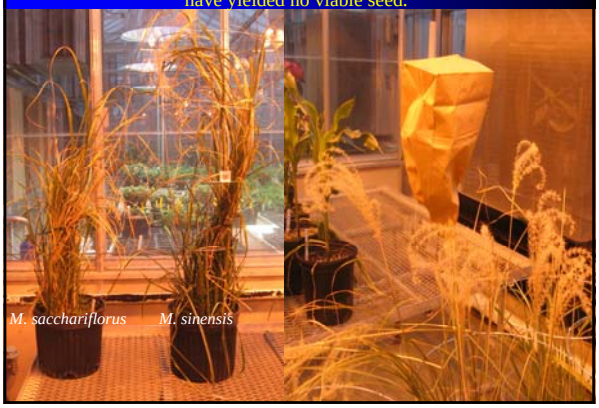
Conventional Breeding

- *M. x giganteus* is a sterile triploid (3N=57) believed to be generated from the hybridization of *M. sinensis* (2N=38) to tetraploid *M. sacchariflorus* (4N=76) (Greef & Deuter, 1993).
- Original genotype collected in Japan and introduced into Europe in 1935.
- Generation of viable seed from this cross is difficult and restricts development of new germplasm.
- Existing *M. x giganteus* lines in Europe show little or no genetic diversity with single genotype accounting for nearly all of the current acreage.

Miscanthus Breeding Program

- Greenhouse hybridizations:
  4 accessions of *M. sacchariflorus* X 10 accessions of *M. sinensis*.
- Field hybridizations will be initiated in 2007.
- Viable seeds will be grown out and plants vegetatively propagated for replicated trials.

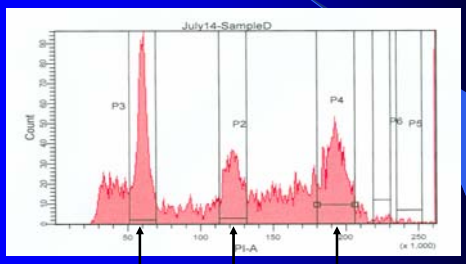
Greenhouse Hybridizations of *M. sinensis* and *M. sacchariflorus* have yielded no viable seed.



Distribution of three Asian *Miscanthus* species

Original *M. x giganteus* hybrid collected in 1935 in Yokohama, Japan, cultivated in Denmark, then distributed throughout Europe and the US as an ornamental plant.

Estimating Plant DNA Content by Flow Cytometry



Corn Sorghum *Miscanthus sinensis*

Plant Regeneration and Somaclonal Variation

- Dr. Jack Widholm has developed a plant tissue culture regeneration system for *M. x giganteus.*
- Over 90 of these tissue-culture generated plants have been transplanted into field plots.
- Preliminary phenotypic evaluation of these plants conducted in the summer and fall of 2006.

Miscanthus Chromosome Doubling

- Chromosome doubling has been associated with increased rates of plant biomass accumulation (sugar cane and corn).
- Mr. Khalid Ibrahim is now in the process of doubling the cell chromosome number in *M. x giganteus, M. sinensis, and M. sacchariflorus* using colchicine and oryzalin treatments to create polyploid plants.

Miscanthus Chromosome Doubling

Immature Inflorescense

Colchicine and oryzalin Treatment of explants for 2, 4, and 6 days

Callus on regeneration media

Regeneration of polyploid *M. sinensis*, *M. sacchariflorus*, and *M. x giganteus*

Ploidy level of calli determined by Flow Cytometry

Gene Transformation Systems

- Due to the difficulties associated with conventional breeding for *Miscanthus* we are collaborating with Dr. Widholm's lab to apply genetic engineering technologies for *Miscanthus* genetic improvement.
- Mr. Hyoung Seok Kim is developing a *Agrobacterium* - mediated *Miscanthus* transformation system.
- Using this system and particle bombardment we propose to transform *M. x giganteus* with candidate genes to provide tolerance to the herbicide PPT.

Agrobacterium-mediated transformation of *Miscanthus*

***Miscanthus* immature inflorescence tissue**

Callus induction on selection medium containing herbicide

Production of embryogenic callus

***Agrobacterium* infection**

Production of transgenic *Miscanthus*

Shoot regeneration

Selection for *Agrobacterium*-mediated Transformants with Herbicide Resistance

Callus transformed with *Agrobacterium* cells harboring pCAMBIA3301 grows on selection medium containing phophinothricin (PPT).

Non-transformed immature inflorescence tissue did not form callus on selection medium.

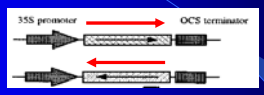
Future Applications of *Miscanthus* Gene Transformation

- Use of Antisense and RNAi gene silencing in *Miscanthis x giganteus* to:
  - a) Modify stalk cellulose/lignin composition ratio to reduce enzymatic pretreatment and increase biomass conversion to ethanol.
  - b) Enhance and prolong vegetative growth by reduced expression of inflorescence induction genes.

Transformation Technology for Candidate Gene Suppression in *Miscanthus*

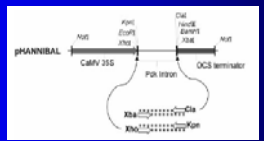
Sense construct

Antisense construct



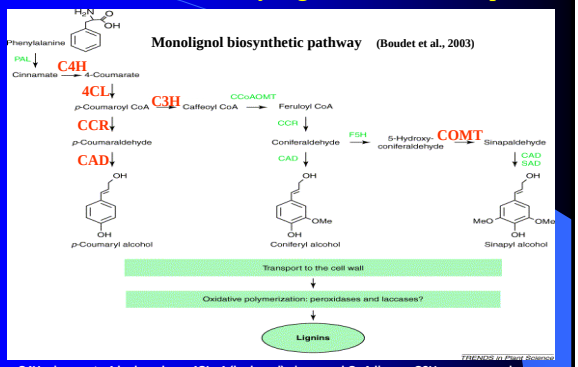
RNAi construct

(Wesley et al., 2001)



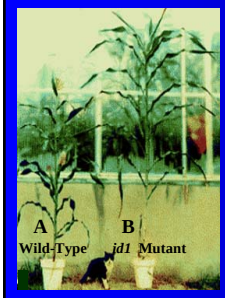
The application of post-transcriptional gene silencing (PTGS) using antisense or RNAi gene constructs will be employed to generate differences in candidate gene expresssion in transgenic plants.

Candidate Genes to Modify Lignin/Cellulose Composition



C4H, cinnamate 4-hydroxylase; 4CL, 4-(hydroxyl) cinnamoyl CoA ligase; C3H, *p*-coumaroyl shikimate/quinate 3'-hydroxylase; CCR, cinnamoyl CoA reductase; CAD, cinnamyl alcohol dehydrogenase; COMT, caffeic acid/ 5-hydroxyferulic acid O-methyltransferase

Candidate Gene for *Miscanthus* Transformation to Delay Flowering



Comparison of Wild-Type Maize with homozygous *indeterminate1* (*id1*) mutant (A).

Normal plant on the left (*Id1*) has 13 leaves, a normal tassel, and two ears; the homozygous *id1* mutant (right) with same genetic background has 20 visible leaves and shows no signs of flowering (B).

***id1* mutant developed normally but produced many more leaves with greater biomass accumulation than wild type maize (Colasanti et al., 1998).**

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