Environmental and Regulatory Sustainability of Genetically Engineered Bioenergy Feedstocks

The case of switchgrass

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Biomass utilization is a multifactorial problem

Land
- Current cropland
- Fallow land (CREP, etc)
- Marginal land
- Rangeland
- Forestland

Feedstocks
- Grain crops
- Softwood
- Switchgrass
- Animal wastes
- Agric. residues
- Bagasse
- Oil crops
- Other

Process technologies
- Fermentation & Enzymes
- Pulping
- Gasification
- Thermo/chemical conversion
- Anaerobic digestion
- Other

Output/Products
- Chemicals
- Ethanol
- Butanol
- Diesel
- Wood products
- Polymers
- Gas fuels: methane, H2, syngas
- Ash, fertilizers

Brian Davison, ORNL
Difference between petroleum and bioenergy feedstocks

http://www.energyinst.org.uk/education/coryton/images/column.gif
Corn vs. cellulosics

Corn

- Endosperm
- Seed Coat (Pericarp)
- Cotyledon (scutellum)
- Aleurone
- Coleoptile
- Plumule leaves
- Shoot Apical meristem
- Root Apical meristem
- Coleorhiza

160 bu/acre = 4.5 tons/acre

Switchgrass

12 tons/acre
Identify, Understand and Manipulate the Plant

Biomass Formation and Modification

Characterization and Modeling

Biomass Deconstruction and Conversion

Switchgrass

Plant Cell Wall

Populus

Lignin  Hemicellulose  Cellulose

Enzymes

Microbe

BioEnergy Science Center
Bioenergy and plant genomics: Expanding the nation’s renewable energy resources

Yesterday

Conventional Forestry

Today

Short rotation hardwoods

Whole Genome Microarrays

Metabolic Profiling

Carbon allocation

Accelerated Domestication

Tomorrow

High yield wood crops

Brian Davison ORNL

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Brian Davison ORNL
Cell wall structure

Switchgrass biotechnology goals

• Enable high-throughput transformation
  – Tissue culture system
  – Transient expression tools
  – Stable transformation system
  – Vectors for genes of interest
• Altering cell wall biosynthesis/modified lignin
• Transgenic plant-expressed cellulases and ligninases
• Increased yield/domestication
• Field performance
• Biosafety/biocontainment
• SUSTAINABILITY
Plant cell wall and membrane

Plant Cell

Nucleus

chromosome

T-DNA integrated into plant chromosome

T-DNA

Ti plasmid

Agrobacterium tumefaciens
Immature inflorescences from top internodes are collected from the greenhouse.

Sterilization & dissection

Half internodes plated on Phase I Medium

2 Weeks in growth chamber

~1.5 cm long pieces plated on Phase II Medium (Callus Induction)

Embryo development: Phase III

~2 Months transfer calli

Established Callus

4 Weeks in dark

Somatic embryogenesis

Rooting medium: Phase IV

30 Days in growth chamber

~14 Days

Established plants

Summary

- On average, 1 half-internode of Alamo-2 has the potential to regenerate 40 whole plants to soil in 4 months, whereas ST1 can regenerate 17 whole plants.
Improvement of tissue culture and transformation systems

- Reduces transformation procedure by 2 months
- Higher efficiency

Proliferating callus

After 2-6 weeks place on rooting to root.

After 2 weeks place onto regeneration to form shoots.

Agrobacterium-mediated transformation

Pick transgenic callus after two months on selection.
What biomass crops where?

Lynn Wright et al., ORNL
But switchgrass is not the perfect choice

• Tailored feedstocks for needs
• Differences in adaptation
• Resource base
• Geographic and regulatory considerations
Disadvantages

• Stand establishment
• Lower yields than Misc.

• Annual
• Inputs
• Bad candidate-biotech

• Vegetative propagation
• Low genetic variation
• Agronomy

• Adaptation-cold
• Vegetative propagation
• Inputs
Potential biomass of switchgrass

Dry Tons of Switchgrass

- Zero
- Zero to 300 thousand
- Up to 1 million
- Up to 2 million
- Over 2 million

Daniel de la Torre Ugarte
Ideal bioenergy feedstock?

✓ • Widely adapted
✓ • High yield
  • Low inputs
  • Not recalcitrant to digestion and processing
  • Homogeneous/canalized traits
✓ • Stress tolerant
  • Farmer friendly
  • Economically friendly
  • Ecologically friendly

Or recipe for guaranteeing invasiveness?
### Platforms

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>NEB GJ/ha/yr</th>
<th>NER</th>
<th>CO₂ Balance</th>
<th>Annual</th>
<th>Establishment</th>
<th>Germplasm</th>
<th>Ag Practice</th>
<th>Ecological Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethanol from starch or sucrose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>10-80</td>
<td>1.5-3.0</td>
<td>Positive</td>
<td>Yes</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Sugarcane</td>
<td>55-80</td>
<td>3.0-5.0</td>
<td>Negative</td>
<td>No</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Sugar beet</td>
<td>40-100</td>
<td>2.5-3.5</td>
<td>Positive</td>
<td>Yes</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Sorghum - sweet</td>
<td>85-300</td>
<td>5-10</td>
<td>Positive</td>
<td>Yes</td>
<td>+++</td>
<td>++</td>
<td>++</td>
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<tr>
<td><strong>Ethanol from Cellulosic feedstock</strong></td>
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<tr>
<td>Miscanthus</td>
<td>250-550</td>
<td>15-70</td>
<td>Negative</td>
<td>Yes/No</td>
<td>+</td>
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<td>+</td>
<td>+++</td>
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<tr>
<td>Switchgrass</td>
<td>150-450</td>
<td>10-50</td>
<td>Negative</td>
<td>No</td>
<td>+</td>
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<td>+++</td>
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<td>Poplar</td>
<td>150-250</td>
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<td>Negative</td>
<td>No</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
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<td><strong>Biodiesel</strong></td>
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<td></td>
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<tr>
<td>Soybean</td>
<td>-20- 10</td>
<td>0.2-0.6</td>
<td>Positive</td>
<td>Yes</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Canola</td>
<td>-5 – 2</td>
<td>0.7-1.0</td>
<td>Positive</td>
<td>Yes</td>
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<td>03-0.9</td>
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<td>+++</td>
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So, biotechnology could be a bioenergy game changer… what about regulations and public acceptance?

- Biotech food crops still have issues of acceptance and regulations
- But we don’t eat dedicated energy crops
- Special problems with transgenic perennials
- Special problems with transgenic plants grown in their geographic center of diversity
- Gene flow is still a regulatory train wreck
Biotech tools to mitigate transgene flow: biocontainment

- Transgenes on chloroplasts
- Transgenic mitigation: tandem constructs
- Site specific recombination or zinc finger nuclease
- Tissue specific apoptosis → male sterility

Focus on limiting gene flow via pollen
GM gene deletor

Chopping transgenes out of pollen
**Gene deletor**

(Luo et al. 2007 Plant Biotechnol J 5:263)
Gene deletor
(Luo et al. 2007 Plant Biotechnol J 5:263)

No recombinase vector

Cre-loxP/FRT vector

![Diagram of gene deletor systems](image)
Tissue-specific apoptosis

Killing pollen cells before they can pollinate
Agroinfiltration—a means of rapid assessment of gene expression
Agroinfiltration—marker gene
Power T via agroinfiltration
Tissue specific apoptosis
Conclusions

- The choice of feedstock is critical—no clear perfect choice, but lots of ways to go wrong
- Switchgrass will benefit from biotechnology
- Switchgrass tissue culture system and transformation tools are available
- Regulatory concerns: gene flow and controlling gene flow are both important
- Transgenic switchgrass will require biocontainment for deregulation
- Several biocontainment tools are available
- We must learn from our past mistakes
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