ENERGY CROP DEVELOPMENT THROUGH GENETIC ENGINEERING

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GAME PLAN

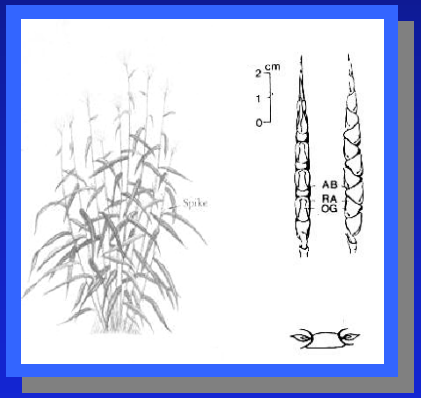
- Domestication in general
- Examples of candidate genes
- Some recent progress
- A new partnership emerging
- The need for flowering control
- Future research needs
- Summary

CORN DOMESTICATION

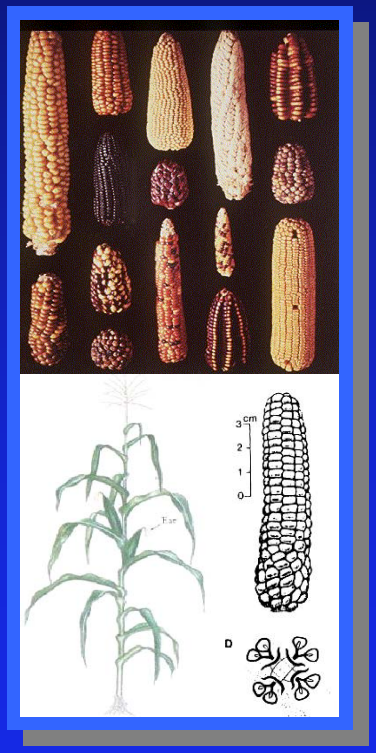
5,000 ybp — 2,000 ybp — Today

Timeline

Teosinte



Indian Corn

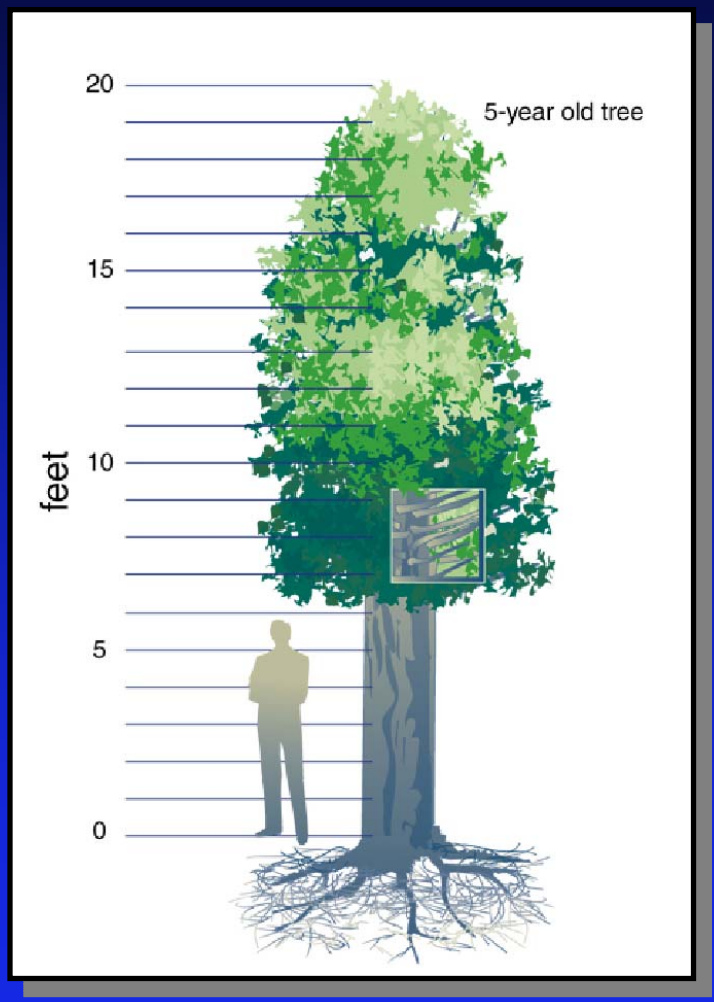


Modern Hybrids

- Mutations in just 5 genes
- Trees harder to domesticate via conventional means

DOMESTICATED *POPULUS* ATTRIBUTES

- Greater carbon allocation to stem (or roots)
- Reduced height growth
- Optimized response to light (competition, proleptic branching)
- Less extensive root system
- Adventitious root formation
- Improved wood chemistry
- Pest resistance
- Flowering control
- Phytoremediation



EXAMPLES OF OTHER CANDIDATE DOMESTICATION GENES

Gene Name	Gene Function	Species
PHYB	competition response	*Arabidopsis*
tb1	lateral branching	maize
GAI	height growth	rice
FLC	delayed flowering	*Arabidopsis*
ABI1	dormancy	*Arabidopsis*
Revoluta	stem thickness	*Arabidopsis*
Pt4CL1	lignin content	aspen

CARBON SEQUESTRATION

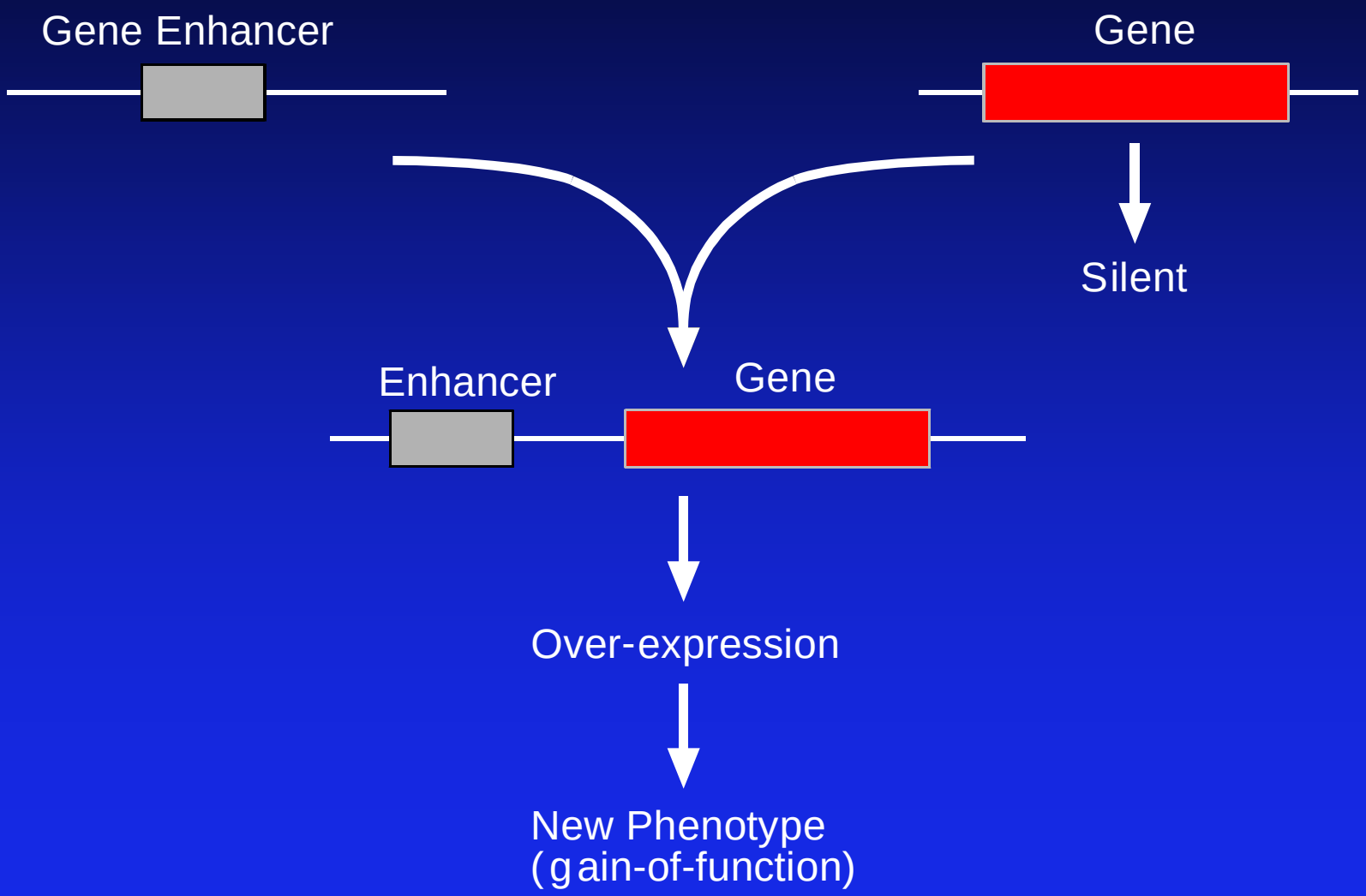
- An existing project funded through DOE's Terrestrial Carbon Sequestration program
  - "Genome-enabled Discovery of Carbon Sequestration Genes in *Populus*"; S.H. Strauss, R. Meilan, and A.M. Brunner
  - Linked to two other proposals (UFL, ORNL)
- Key aspects: Allocation and Partitioning
  - Redirect carbon to below-ground tissues
  - Emphasizing genes in pathways involved with auxin and cytokinin
  - Store carbon in compounds more resistant to degradation (*e.g.*, phenols, lignin)
- Poplar as a model
  - Rapid growth, small genome that is entirely sequenced; easy to transform, regenerate, and vegetatively propagate; many well-defined pedigrees, developmentally plastic, EST databases

GENES AFFECTING STATURE AND BRANCHING

- **Phytochrome genes involved in sensing competition**
  - **Mutants (insensitive) allocate more carbon to the stem**
  - **We have transformed constructs designed to selectively silence, through RNAi, each of poplar's two *PHYB* genes**
  - **Into female hybrid aspen INRA 717-1B4 (*P. tremula* x *P. alba*)**
  - **>40 lines per construct (all PCR verified)**
  - **4 ramets/line will be field tested in OR beginning Spring 2004**
- ***GAI* and *RGL1* involved in regulating gibberellic acid (GA) response**
  - **GRAS family of transcription factors, contain DELLA domain**
  - **Affect stem elongation and flowering**
  - **Wild-type and mutant versions of each transformed into 717-1B4**
  - **Recovered ~10 independent lines/construct**
  - **These will also be field-tested in 2004**

DISCOVERY OF OTHER USEFUL GENES

Activation Tagging



ACTIVATION TAGGING MUTANTS

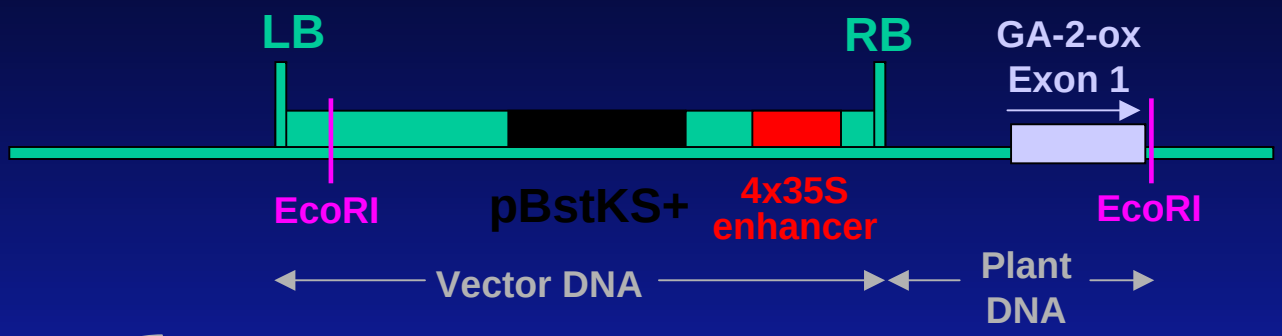




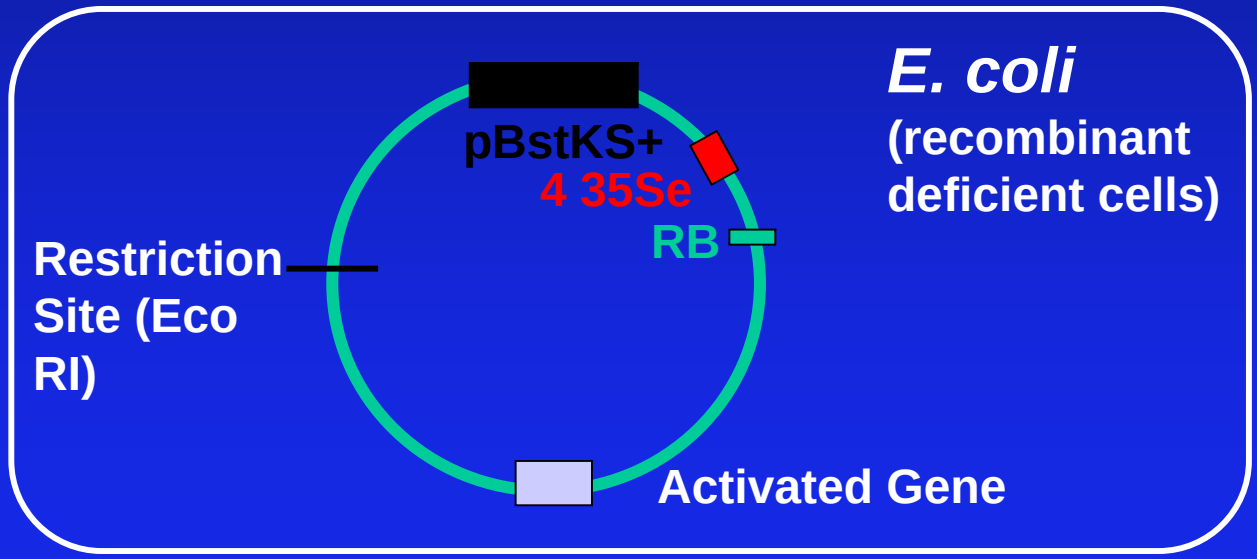


- 629 lines (4 ramets) field-tested to see effects on developmentally delayed genes

PLASMID RESCUE



Restriction + Ligation + Electroporation



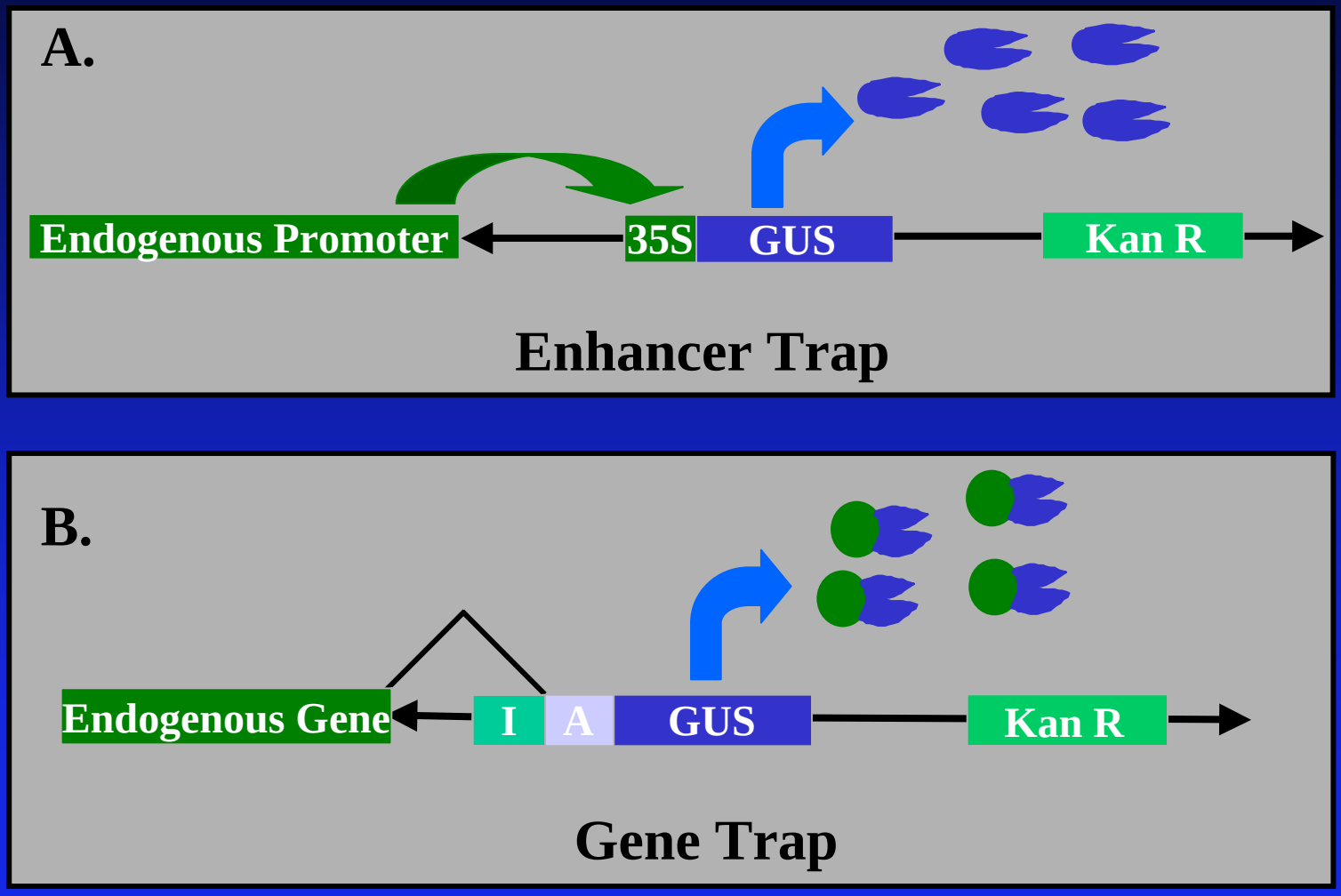
"STUMPY"

- Phenotype
  - Shorter internodes, thick stem, dark leaves
- Characterization done
  - Sequencing
  - GA content (GC-MS)
  - Expression analysis
  - Complementation
- Underway
  - Metabolic profiling (ORNL)
  - Wood properties (NREL)
  - Field test (>2X lines with phenotype)
- Varying expression of this gene will allow us to selectively alter the final stature of a tree

Reference: Busov, V.B., Meilan, R., Pearce, D.W., Ma, C., Rood, S.B., and Strauss, S.H. 2003. Activation tagging of a dominant gibberellin catabolism gene (*GA 2-oxidase*) from poplar that regulates tree stature. *Plant Physiology* 132(3):1283-1291.

ANOTHER APPROACH TO GENE DISCOVERY

Gene and Enhancer Traps



GENE AND ENHANCER TRAPS

- System developed by Andrew Groover
  - Institute of Forest Genetics, Davis, CA
- >1,300 independent lines were produced
  - All produced in INRA 717-1B4 (*Populus tremula* x *P. alba*)
- Andrew screened for vascular expression
  - 125 of 295 enhancer-trap lines exhibited GUS staining
  - 19 of 477 gene-trap lines exhibited GUS staining
  - These numbers exclude common patterns seen
  - Similar to what was seen in *Arabidopsis*
- Why enhancer traps more likely to be successful than gene traps
- We screened for root-specific expression

GENE AND ENHANCER TRAP LINES

Vascular-specific Expression

Enhancer Trap 61-1, photo provided by Andrew Groover

Reference: Groover, A., Fontana, J., Dupper, G., Ma, C., Martienssen, R., Strauss, S., and Meilan, R. 2004. Gene and enhancer trap tagging of vascular-expressed genes in poplar trees. *Plant Physiology* 134: 1742-1751.

GENE AND ENHANCER TRAPS

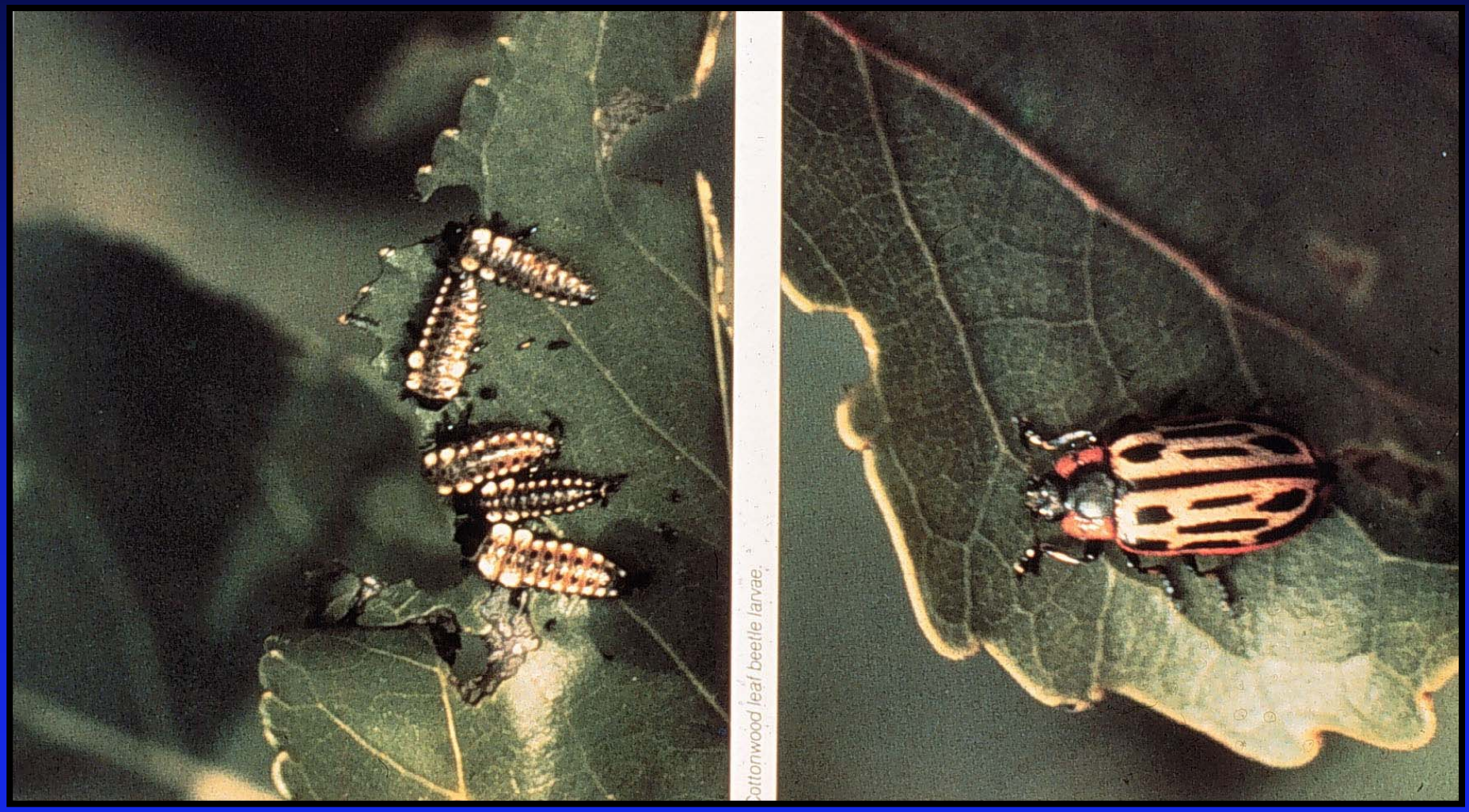
Root-specific Expression

- Stained for GUS activity soon after root initiation
- Sequenced surrounding DNA in 35 independent lines (Barry Goldfarb and Qian Wu, NCSU)
- We'll soon test knock-in and knock-out versions of each
- Functionality test in two genotypes of *Populus*
  - One roots easily *in vitro* and not *ex vitro*; the other behaves in just the opposite manner
  - Want to find genes involved in the control of root initiation.

Photo provided by Barry Goldfarb

COTTONWOOD LEAF BEETLE

Crysomela scripta



ENGINEERED PEST RESISTANCE

Reference: Meilan, R., Ma, C., Cheng, S., Eaton, J.A., Miller, L.K., Crockett, R.P., DiFazio, S.P., and Strauss, S.H. 2000. High levels of Roundup® and leaf-beetle resistance in genetically engineered hybrid cottonwoods. Pages 29-38 *in*: K.A. Blatner, J.D. Johnson, and D.M. Baumgartner, eds., Hybrid Poplars in the Pacific Northwest: Culture, Commerce and Capability. Washington State University Cooperative Extension Bulletin MISC0272, Pullman, WA.

PHYTOREMEDIATION

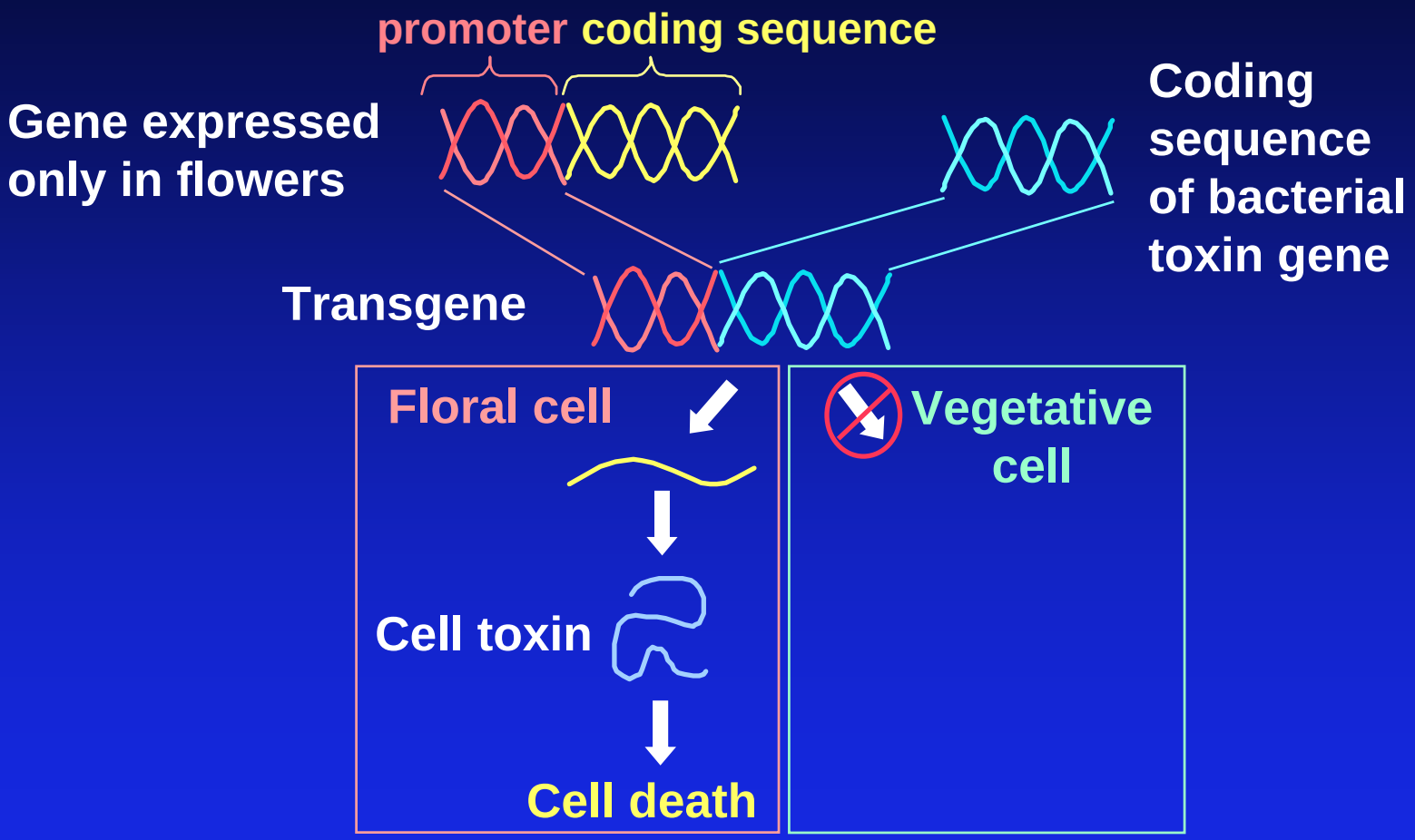
- In collaboration with Milt Gordon and Sharon Doty at University of Washington
- Detoxifying TCE
- Cytochrome P450 2E1 (rabbit)
- We produced 171 transgenic lines (events) in two hybrid aspen genotypes that are now being characterized
- Potential for clean-up of Superfund site(s)

REASONS FOR ENGINEERING REPRODUCTIVE STERILITY

- **Confining transgenes**
  - Assumed to be a regulatory requirement
- **Maintaining rapid growth**
  - Diversion of photosynthate
- **Preventing genetic pollution**
  - From non-transgenic plantations
  - Putting what we do in proper context

ENGINEERING REPRODUCTIVE STERILITY

Ablation (cell death), one of several approaches



Reference

Meilan, R., Brunner, A., Skinner, J., and Strauss, S. 2001. Modification of Flowering in Transgenic Trees. Pages 247-256 in: Molecular Breeding of Woody Plants. Progress in Biotechnology series. A. Komamine and N. Morohoshi, editors. Elsevier Science BV, Amsterdam.

PARTNERSHIP

- HTIRC is in the process of forming a Joint Center with TGERC (OSU) under the NSF I/UCRC program
- TGERC has a 10-year history of flowering-control research using poplar as a model system
- HTIRC and TGERC will collaborate on a flowering-control project for fine hardwoods
  - We will focus on black walnut and black cherry, initially
  - MTAs already in place for obtaining the necessary genetic constructs

RESEARCH NEEDS

- **Transformation**
  - **Develop a protocol that is useful for a wide array of genotypes**
  - **Assemble universal shuttle & binary vector backbones for KI / KO constructs**
  - ***In planta* transformation method similar to the one used with *Arabidopsis***
- **Inducible promoters (for demonstrating functionality)**
  - **Constitutive expression of key regulatory genes may be lethal or have strong negative effects**
- **Large-scale program to alter regulation of all poplar genes**
- **A simple, reliable method of floral induction**
- **A reliable excision system**
  - **Removal of selectable marker genes, to alleviate public concern**
  - **Allow for easy re-transformation**
- **Protocols enabling transgenic stocks to be stored cryogenically at low cost and with low rates of somaclonal variation**

SUMMARY

- Tree domestication provides an approach to improve biomass yield and reduce feedstock costs as part of a larger carbon management strategy
- Genetic engineering produces the same outcome as conventional breeding but in a shorter time frame
- New forward genomics techniques are facilitating gene discovery
- Poplar is a very useful model system for testing candidate-gene functionality
- Flowering control will likely be needed before federal regulators will allow commercial deployment of transgenic trees

ACKNOWLEDGEMENTS

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- Steve Strauss
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- Tree Genetic Engineering Research Cooperative
- National Science Foundation I/UCRC Program
- Agenda2020
- U.S. Department of Energy (Biofuels Development and Carbon Sequestration programs)
- Consortium for Plant Biotechnology Research (CPBR)
- USDA NRICG Program