BiOSentinel Plants: Enabling Farmer Choice

Marie Connett and Richard Jefferson
CAMBIA’s BiOS Initiative:
Biology for Open Society - Biological Open Source
Thank you to the organizers!

- Description of the BiOSentinel concept proposal
- CAMBIA’s current work on modules of the BiOSentinel
- Why “open source” development of these modules?
Thank you to Qifa Zhang, who pointed out that the sector must starting working on “green” agriculture:

- Inputs of pesticides and fertilisers are high cost to farmers
- Overuse is hazardous to health of farm workers and their families
- Significant quantities of these chemicals wash into rivers
If the farmer could know with more precision when inputs are needed, much waste could be avoided.

Key challenges in agriculture, such as wise use of nutrients or protection from pests, are often best solved by management choices of farmers.

These challenges can only be confronted when they are known, and when solutions can be locally chosen.
Effectiveness components of a biological sentinel or bioindicator

- An indicator species suitable for the cultural system, the soil, the climate, etc.
- A receptor for the signal
- Signal transduction and amplification resulting in expression in a part of the plant that is observable
- Translation of the signal into something that can be detected
- A detection system for humans
- Ways to act on the information
Not limited to inputs
Indicator species could detect pollutants or anything that’s difficult to measure directly

Aresa engineered *Arabidopsis* as a biosentinel:
- roots detect TNT residues in the soil that leak from many kinds of land mines
- anthocyanin response in leaves is visible to humans
**Constant biological soils/environment testing**

- An indicator species suitable for the cultural system, soil type, climate, etc.
- A receptor for the signal
- Translation of the signal into something that can be observed
- A detection system for humans

This can be plants outside the food chain but grown alongside the crop.

For a bioindicator of a nutrient deficiency, may need to have expression in the roots of a plant, and signal transduction and possibly amplification.

Example: visible or infra-red color change in leaves, mediated by a GUS gene product, an anthocyanin gene or similar genes.

Expression in a part of the plant that is observable by nondestructive means which are inexpensive or costless to use, and reliable under field conditions (ideally a human’s five senses)
CAMBIA’s current work on modules

- An indicator species suitable for the cultural system, soil type, climate, etc. This can be plants outside the food chain but grown alongside the crop.

➢ Locally appropriate choices of species easy to grow and that won’t interfere with harvest practices

Engineering these will require a widely usable, broadly applicable transformation system
- Many explant types are recalcitrant to regenerate following exposure to conditions suitable for *Agrobacterium*
- Freedom to operate using *Agrobacterium* is not universal; hundreds of patents
Why was TransBacter™ developed?

The science

Gene transfer to plants in nature may not be limited to *Agrobacterium*

- *Agrobacterium* evokes plant responses typical of a pathogenic infection, causing plant stress, genomic consequences and regeneration challenges.

- Co-evolution of plant pathogenic capacity and plant response may have unduly restricted host-range, susceptible plant cell types.

- Using plant symbionts, commensal and endophytic bacteria could greatly expand our options.
General strategy for the TransBacter project

A. tumefaciens

- Ti plasmid mobilisation
- GusPlus™ binary vector

pTi

Target plant cell

- Tobacco
- Rice
- Arabidopsis

Other bacteria

- Sinorhizobium meliloti
- Rhizobium sp.
- Mesorhizobium loti

T-DNA

Condition optimisation
Transbacter-mediated rice transformation

Stages of rice transformation process, showing GUSPlus activity

With Transbacter use in multiple bacterial species, CAMBIA’s small lab team has produced hundreds of independent transgenic rice, tobacco and *Arabidopsis* plants.
CAMBIA’s ongoing improvements to Transbacter

- Extension to more different species of bacteria
  - Greatly reduce size of Ti plasmid
  - Broad host range origin of replication
  - Inducibility of portions of vir operon

- Simple BiOS-compliant materials transfer agreement ([www.bioslicense.net](http://www.bioslicense.net)), spot plasmid and mail
**BiOS-compliant Agreements**

If you execute a BiOS license or accept a BiOS MTA:

- You are granted the right to use the technology to do research, improve the technology, AND make products.
- In return, you agree not to use IP rights to prevent others from doing the same.
- And you agree to sharing biosafety information and improvements constituting enabling technology with the community for common good.

Compare the normal practices through millenia of agriculture…
**BiOS-compliant Agreements**

If you execute a BiOS license or accept a BiOS MTA you have the right to do research AND make products:

- There is no “research” license. “For research use” restrictions are too often used to capture research while stopping the choice of partners for commercialisation ("‘free of charge’ does not mean ‘free of cost’" -- Prof. Brian Wright, Agricultural Economics, UC Berkeley)

- Licensed Products can be made, offered for sale, sold, and used
  - For further research
  - In internal operations, for example to save money
  - For public good
  - Or in order to make a profit
BiOS-compliant Agreements

If you execute a BiOS license or accept a BiOS MTA, you are granted the right to use the technology

➢ and in return, you agree that the technology may be shared and you may not use IP rights to prevent others from doing the same: “open source”
Ten years ago “open source software” was feared by many of the same companies that use it now for most of their technology, patented or not!

By using open source enabling tools, startups are making millions of dollars on applications: Google

CAMBIA’s current work on modules

- A receptor for the signal

For a bioindicator for a nutrient deficiency, may need to have expression in the roots of a plant, and signal transduction and possibly amplification.
**Distribution of 100 Blast Hits on the Query Sequence**

Mouse-over to show delve lines and scores. Click to show alignments

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**Note that these are “patent pending”**
1: AR907030. Reports Sequence 5 from p...[gi:112085625]

Features  Sequence

LOCUS   AR907030
DEFINITION Sequence 5 from patent US 7078234.
ACCESSION AR907030
VERSION AR907030.1 GI:112085625
KEYWORDS
SOURCE Unknown.
ORGANISM Unknown. Unclassified.
REFERENCE 1 (bases 1 to 21)
AUTHORS Kris, A.L. and Voylis, D.A.
TITLE Maize embryo-specific promoter compositions and methods for use thereof
Monsanto Technology LLC; St. Louis, MO; US;
REMARK CAMBIA Patent Lens: US 7078234
FEATURES Location/Qualifiers
source 1..21
//
organism="unknown"
/mol_typed= "genomic DNA"

1 ggcgcgtcoca aactaagag c

//

Disclaimer | Write to the Help Desk
NCBI | NLM | NIH
Maize cDNA seq claimed by Ceres in 2003 (and sequences >65% similar)

87% alignment to sorghum 2005 public seq
“After-claiming”

“There are many different ways to “claim” an invention in a patent application…then file continuations to obtain coverage for other aspects.

That practice is fine and legal, but can create business problems when the applicant waits until learning of a competitor product before adding claims that cover that new product…[that] has arisen in the marketplace since filing the original patent application… the possibility of late claiming creates another potential down-the-road liability for product developers.”

US patent application 20060150283 by Ceres (filed 2004) claims an isolated nucleic acid molecule comprising:

a) a full length cDNA nucleic acid having a nucleotide sequence which encodes an amino acid sequence exhibiting at least 40% sequence identity to an amino acid sequence encoded by

(1) a full length cDNA nucleotide sequence described in the Sequence Listing or the Sequence Listing-Miscellaneous Feature documents, or a fragment thereof; or

(2) a complement of a full-length cDNA nucleotide sequence shown in the Sequence Listing or the Sequence Listing-Miscellaneous Feature documents, or a fragment thereof;

b) a nucleic acid which is the reverse of the nucleotide sequence according to subparagraph (a), such that the reverse nucleotide sequence has a sequence order which is the reverse of the sequence order of the nucleotide sequence according to subparagraph (a);

c) a nucleic acid capable of hybridizing to a nucleic acid having a sequence selected from the group consisting of: a full-length cDNA nucleotide sequence which is shown in the Sequence Listing or the Sequence Listing-Miscellaneous Feature documents; and a nucleotide sequence which is complementary to a full-length cDNA nucleotide sequence shown in the Sequence Listing or the Sequence Listing-Miscellaneous Feature documents, under conditions that permit formation of a nucleic acid duplex at a temperature from about 40°...
>2000 seqs claimed; >70 apps in this family dating from 1999
BiOSentinel modules, continued

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- A receptor for the signal

- Translation of the signal into something that can be observed

This can be plants outside the food chain but grown alongside the crop.

For a bioindicator for a nutrient deficiency, may need to have expression in the roots of a plant, and signal transduction and possibly amplification. Many different signal molecules possible.

Visible or infra-red color change mediated by a GUS gene product, an anthocyanin gene or similar genes.
Both GUSPlus and CAMBIA’s transactivation constructs are freely available under the BiOS license.
Legal instruments to support new norms

To draft BiOS-compliant licenses we pulled input from

- Corporate counsels
- University tech transfer attorneys
- Private patent attorneys
- Prospective licensees

A BiOS-compliant license can be used to make available any technology that is subject to patent and other IP rights. Other BiOS-compliant agreements can cover biological sequence information and materials.
This work was supported in part by grants from the Lemelson Foundation and the Rockefeller Foundation, and by the Ministry of Foreign Affairs of the Government of Norway through a grant to the International Rice Research Institute.