



ISB NEWS REPORT

COVERING AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY

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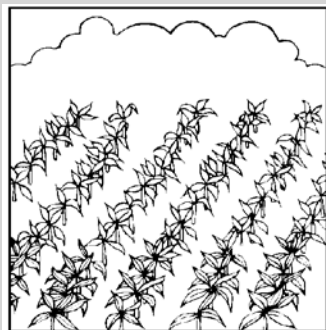
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EMERGING TECHNOLOGIES

Emerging Plant Biotechnologies: New Bugs for Old

Zac Hanley, Kieran Elborough

One species of creativity is the fruitful combination of concepts from different fields. Ideas imported from without can catalyse great changes in science—physicists and chemists helped biologists to become molecular biologists, while mathematicians co-parented bioinformatics into existence. Now one agri-biotech organization wants to enliven the industry with the clever, creative combination of an updated biotechnology and a decades-old idea from computer programming.

Black boxes and building bricks

Biotechnology is the redeployment of natural systems for human ends; molecular biology is the artful arrangement of life's little black boxes. Scientists borrow systems, processes, and architecture built and honed by natural selection for millennia. We characterize their function and activity then re-arrange and re-purpose them. Genes, regulons, proteins, plasmids, chromosomes, bacteriophages, cells, tissues, organs, and whole organisms are treated as 'black boxes', i.e., modules that perform a function but for which the contents may for the moment remain mysterious. Functional modules are useful because we know *what* they do, whether or not we know exactly *how* they do it. Restriction endonucleases, for example, are treated as embodiments of their specific activity—even in the hands of a novice, *EcoRI* or *XhoI* represent black boxes which can be given inputs (DNA substrate, buffer, ions) and predictably deliver certain outputs (DNA cleaved at the *EcoRI* or *XhoI* recognition sites). Utility is, here, everything; the detail of what is inside the box is secondary and can be left to those on other grants. The boxes are also building bricks or line-replaceable components—like Lego® or military aircraft parts—because they can be switched and swapped for others that behave similarly. For such swaps, each 'box' may have different contents: the isoschizomer *TliI* can, in most cases, replace *XhoI*, and many diverse polymerases are available for PCR.

Much of molecular biology (*in vitro* and *in vivo*) is like this. Research scientists open life's black boxes to find out how an endonuclease, polymerase, or T₄ bacteriophage works. They often find smaller black boxes nested inside. This simplistic depiction of molecular biology is aptly reductionist; genes construct, and are themselves constructed of, switchable modules too; that is Life. This reductionism can lead to cognitive biases; it is also incontestably successful.

Agrobacterium tumefaciens, described in *Science* as "Nature's genetic engineer"¹, is the vehicle for the only known example of natural trans-kingdom horizontal inheritance. Widely used in agricultural biotechnology, Agrotransformation also



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showcases modern biotechnology as the artful placement of Nature's marvellous modules and scientific research as vigorous box-opening that provides answers and nested black boxes. Some examples:

- The fundamental conceptual breakthrough in plant genetic engineering was the realization that most of the heritable material trafficked to the plant by *Agrobacterium* was not essential to the process. Opening this particular black box (the T-DNA) revealed several components, only some of which were prerequisites. Any gene could be introduced if flanked by left and right border regions because the wild type opine synthesis genes were replaceable components.
- The limited host range of wild type *Agrobacterium* was shown to arise from its preference for (taxonomic) class-specific biochemicals released in response to tissue damage. Opening this box allowed investigators to source this particular component differently, supplying equivalent conditions to other plants and tissues *in vitro* and thereby increasing Agrotransformation efficiency and broadening the host range.
- Problematic manipulation of the Ti plasmid gave way to widespread use of binary vectors when research revealed more potential for swaps and shuffles. The plasmid 'black box' turned out to be made up of the T-DNA and several *vir* genes encoding proteins involved in its transport; these proteins, being diffusible within the cell, function *in trans*, i.e., they need not be located on the same plasmid as the T-DNA. These smaller 'black boxes' (some *vir* protein functions are still obscure) were combinatorial. Binary vectors comprise a pair in which a partially disarmed Ti plasmid, unable to mobilize its own T-DNA, facilitates DNA transfer to the plant from a second, more manipulable vector.

The omitted box

Broothaerts and co-workers at the Centre for the Application of Molecular Biology to International Agriculture (CAMBIA) report that another element of the Agrotransformation process is replaceable². This component has been overlooked by some, perhaps due to the aforementioned cognitive biases since it is too big, and perhaps ignored by others for practical reasons. Nevertheless, it is a switchable, swappable module in the tradition of molecular biology: it is the *Agrobacterium* itself, just one more 'black box' that can be substituted by other boxes with analogous input-output characteristics. *Agrobacterium* is, it seems, unnecessary for Agrotransformation.

When these workers introduced a typical binary plasmid system into three isolates of plant-associated bacteria, all proved capable of transforming tobacco. All were nodulating symbionts associated with legume root systems, namely the promiscuous nodulator *Rhizobium* NGR234, the alfalfa symbiont *Sinorhizobium meliloti*, and *Mesorhizobium loti*. Collectively, the inventors refer to these species as TransBacter™. Full genome sequences are available for *Sinorhizobium*, and *Mesorhizobium*³. The binary-equipped *Sinorhizobium* species was also used successfully to transform *Arabidopsis* (via floral dip) and rice callus. For the first time, molecular evidence of trans-kingdom



horizontal transfer mediated by non-*Agrobacterium* bacteria is available. These bacterial species are not all obvious choices: *Rhizobium* is con-generic (some claim con-specific) with *Agrobacterium* but the others are much more distantly related. For the removal of doubt, constructs used in non-*Agrobacterium* species were designed to be diagnostically different in restriction analysis and sequence-specific PCR, while the bacteria themselves were genotyped and maintained under selective conditions to prevent *Agrobacterium* contamination.

This paper is just a preliminary report. A scant three plant species were transformed and only *Sinorhizobium* could transform all three; yet note that these three plant species are two excellent dicot models for further technique refinement and one monocot that is the dietary staple of over two billion people. Only a few transformants were produced, handfuls among hundreds; yet note that early success rates for new protocols are rarely impossible to improve. The idea itself is not novel and the authors faithfully report that transfer of (Ti plasmid-mediated) virulence *A. tumefaciens* to avirulent *Agrobacterium* species was first published over half a century ago⁴; yet note that they present the first unambiguous molecular evidence of a functioning and modular system ready to be taken up by biotechnologists everywhere. Lastly, a mere two generations of transgenic plants have been followed; yet note that the expected and heartening Mendelian segregation of the transgenes has occurred in all three plant species.

Free, as in speech

This breakthrough is more than a demonstration of academics at their most artful playing with nature's parts list, or confirmatory evidence of a fifty year old research paper. The authors state early on that one reason for the work was that "the complexity of the patent landscape has created both real and perceived obstacles to the effective use of [Agrotransformation] technology for agricultural improvements." Their paper is the sketch of a map for a path around these obstacles, a path still a little overgrown and as yet unpaved. The obstacles consist of complex contracts and licenses for the use of patents held on Agrotransformation methods, for the binary vector concept, and covering many other components of the process as commonly practiced. CAMBIA is offering alternatives to what some have seen as an oligopoly in key agricultural biotechnologies.

Their Biological Innovation for Open Society (BIOS)⁵ initiative seeks to create a biotechnology commons, a suite of tools and information not bound by contractual arrangements currently offered by holders of strategic intellectual property in agricultural biotechnology. Researchers and companies may join the associated community, known as BioForge,

and access TransBacter species and other technologies held in commons. To do so, community members abide by an unusual contractual relationship modelled on the computer programming community's long-established open source licenses such as the 'copyleft' system. These are justly celebrated as having facilitated the development of huge, dispersed, successful projects like the GNU-Linux operating system and Wikipedia⁶. Software developed and shared under such licenses is 'free', where 'free' is clarified by their catchphrase 'free as in speech, not free as in beer'.

BIOS is copyleft's biological orthologue. Members of BioForge may use the technology commons, even sell the resulting products, without paying fees or royalties to the inventors. They may even patent any improvements they make, but, akin to programming copyleft, BIOS members are obligated to make any such improvements, patented or not, available to other members of the community and also share data to assist regulatory or biosafety approvals. The analogy with copyleft and similar arrangements does not survive CAMBIA's insistence on a Technology Support Fee payable to BIOS, although it should be noted that this will be a subscription-like annual payment waived for or payable in kind by non-profit organizations or those headquartered outside the OECD.

The BIOS initiative and the (relatively) free way in which TransBacter is being made available to the world are fragments of a quiet but profound revolution in science. Recent years have seen increased scientific openness; greater efforts at long-overdue public education; wider engagement with the social impacts of science; a renewed and deserved prominence of ethics, accountability, and downstream consequences; and the provision of experimental data and peer-reviewed publications through open access publishing⁸. Conversely and concurrently, the escalating use of strong intellectual property protection massively affects the way the industry develops, from entrepreneurial academics worried about the applicability of the experimental use exception, to opposing transnational companies litigating for decades⁷. All of these factors have forced or are forcing changes on academic research; industry, as always, is downstream. Creative ideas such as the technology commons are now arriving at industry's door with as much or more potential for change. These are exciting times.

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PLANT RESEARCH NEWS

To Carb or Not to Carb — Towards Accessible Low Sugar Sweeteners

Tawanda Zidenga

Carbohydrate seems to have replaced fat as the evil devil in diet talk. While the precise definition is often obscure, the term “low carb ” has now become a catch phrase in supermarkets, magazines, and television commercials. The increasing diet consciousness of modern societies has led a drive towards “low calorie” sweeteners. Low calorie sweeteners are especially important for persons affected by diseases linked to the consumption of sugar, such as diabetes. This drive has naturally increased interest in protein sweeteners as alternatives to carbohydrate-based sweeteners such as sucrose. These low-calorie sweeteners are also called “non-nutritive sweeteners,” “high intensity sweeteners,” or “alternative sweeteners.” An example is thaumatin, a mixture of intensely sweet proteins extracted with water from the arils of the fruit of the West African perennial plant *Thaumatococcus daniellii*. Unfortunately, commercial production of thaumatin, as well as all other sweet proteins, has been limited by the difficulty in cultivating the tropical plant sources of these proteins. Furthermore, repeated attempts to produce recombinant sweet proteins in microorganisms and transgenic plant systems have failed to yield these proteins at sufficiently high levels to make widespread commercialization economically feasible.

Brazzein is isolated from the fruit of *Pentadiplandra brazzeana* Baillon, a plant found in West Africa. It has an intrinsic sweetness 500 to 2000 times that of sucrose and consists of a single chain of 54 amino acid residues and no carbohydrate. The ability to transfer the sweetness to agronomic crops would increase the commercial accessibility of brazzein. Lamphear and colleagues¹ from ProdiGene Inc. have reported the generation of maize lines with high levels of brazzein protein expressed in seed. The fractionation of brazzein-expressing corn seed by standard dry-milling procedures resulted in further enrichment of brazzein in germ flour. Furthermore, brazzein purified from seed was up to 1200 times sweeter than sucrose on a per weight basis. These results support maize as an effective, economical expression system for biopharming the sweet protein, brazzein.

Biopharming

One of the latest trends in genetic engineering work is the production of plants engineered to have proteins or chemicals for pharmaceutical and industrial uses. Plants offer a number of advantages compared to other expression systems. First, they have been shown to express accurately a wide variety of proteins not naturally produced in plants and which undergo complex post-translational processing. Secondly, plant-based production can be scaled up by capitalizing on bulk production of plants. In addition, plants do not harbor any known human pathogens, thus minimizing the fear of harmful contaminants. Finally, the expression of proteins in seeds provides a natural site for the long-term storage of proteins¹. Edible crops have been used to deliver oral vaccines via biopharming approaches. Maize, having a well-developed commercial agricultural system worldwide, is a particularly suitable vehicle for the commercial production of consumable proteins.

In expressing brazzein protein in maize, the researchers focused on increasing the level of expression while trying to generate an expression product structurally and functionally equivalent to the protein from its natural source. Only type 1 and type 3 forms of brazzein are detected in the ripe fruit of *P. brazzeana* and thus the goal was to produce these forms through a recombinant plant expression system. They achieved the highest expression when they expressed brazzein using an embryo-preferred promoter and targeting it to the cell wall. The high expression of brazzein protein in corn seed embryo gave a germ fraction with an intrinsic sweetness of up to 40% of the value of sucrose by weight using a seed pool with an expression level of only approximately 50 µg type 3 brazzein per gram seed. Lamphear *et al.* project a potential for unfractionated whole grain to exceed the sweetness



INDUSTRY NEWS

Retaining Genetically Engineered Seed? Consider Retaining a Lawyer

Phillip B.C. Jones

“My daddy saved seed. I saved seed,” Homan McFarling recently told *The Associated Press*. The Mississippi farmer has been fighting Monsanto in federal courts over his right to replant crop seed derived from a harvest. While the practice of saving seed may be ancient, the seed itself is something new. It contains Monsanto’s proprietary technology.

Federal Circuit Upholds Prohibition against Saving Seed

In 1998, McFarling purchased Monsanto’s Roundup Ready® soybean seeds and signed the company’s Technology Agreement. This contract required him to use the seed for planting a commercial crop in a single season. It also recited three prohibited acts: supplying seed to any other person for planting, saving any crop produced from the seed for replanting, and supplying saved seed to anyone for replanting. Despite the prohibitions, McFarling saved 1500 bushels of Roundup Ready soybeans from his 1998 harvest and planted them the following year. He saved over 3000 bags of soybeans from his 1999 harvest for his next crop.

Before McFarling replanted soybeans collected from the 1998 harvest, he sent them to a third party for cleaning. Monsanto obtained a sample and had the DNA analyzed at Mississippi State University. When genetic analysis indicated that McFarling had saved Roundup Ready seeds, Monsanto sued the farmer in the Eastern District of Missouri, alleging patent infringement and breach of contract. The court forbade the farmer from using seed saved from crops grown with the patented soybeans. McFarling appealed to the Court of Appeals for the Federal Circuit. He lost.

Back in district court, Monsanto moved for summary judgment on its claims for patent infringement and breach of the Technology Agreement. The court ruled in favor of Monsanto and McFarling appealed. Once again at the Federal Circuit, McFarling argued, among other things, that the district court erred when it ruled against his patent misuse defense, his antitrust counterclaim, and his defense under the Plant Variety Protection Act.

The patent misuse doctrine aims to prevent a patentee from suppressing competition by impermissibly broadening a patent’s scope. McFarling argued that Monsanto had committed patent misuse by impermissibly tying an unpatented product to a patented product. He argued that “[b]y

intensity of sucrose, based on the capacity to increase brazzein expression levels through continued backcrossing of lines into elite inbred germplasm and the generation of homozygous hybrid parent seed for production.

Applications

One potential application of brazzein is as a refined food additive. To determine whether the purified material retains high intrinsic sweetness, a multistep purification scheme for the isolation of brazzein from maize seed was used. Brazzein expressed in maize possessed the same sweet phenotype as the natural product. This is unlike thaumatin, where several approaches using other expression systems yielded recombinant protein that was not sweet. Brazzein-containing germ flour can be used directly in potential product applications without further enrichment to supply sweetness and improved product flavor, showing the potential of this unpurified material as a low-intensity sweetener. Lamphear *et al.* believe that using a germ fraction for product sweetening not only provides a low-calorie alternative to sucrose, but also gives the intrinsic bulking properties necessary to replace the volume lost on removal of sugar, yielding a combined sweetener and bulking agent. The paper further speculates on possible applications of corn-produced brazzein, including using brazzein from corn to enrich a high-fructose corn syrup fraction to generate a formulation with increased sweetness.

The bigger picture

Biopharmaceutical crops are the subject of much controversy, with critics arguing that they are not worth the risk, while biotechnologists believe they could, if properly applied and regulated, contribute to cheaper production of drugs and other industrial products. Commercialization of the technology will require regulatory approval. Patent protection of brazzein has been debated in previous years, as some believe that the indigenous communities that have been using it for ages deserve compensation from its commercialization. The paper summarized in this report however provides support for a promising technology with increasing applications of biotechnology research beyond the farm.

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prohibiting seed-saving, Monsanto has extended its patent on the gene technology to include an unpatented product—the germplasm—or God-made soybean seed which is not within the terms of the patent.” The court did not accept this argument, because McFarling could buy soybean germplasm without the glyphosate tolerance trait that brings the soybean within the scope of Monsanto’s patent.

The court looked beyond this tying argument and suggested that something else really annoyed the farmer: the license controlled what McFarling could do with the second generation seeds that he had produced with the seeds acquired under the license. Monsanto’s patent entitles the company to this degree of control, the court reasoned, because the patent claims cover the second generation of Roundup Ready soybeans. With regard to McFarling’s antitrust argument, the court considered this a repackaged patent misuse defense. The court could see no evidence that Monsanto’s licensing restrictions went beyond the boundaries of the patent grant.

In the third line of defense, McFarling contended that the Agreement’s prohibitions violate the Plant Variety Protection Act. The PVPA permits farmers to save seeds of registered plants. The court disagreed that this right under the PVPA imparts the right to save seeds protected by the Patent Act.

Several months after the *McFarling* decision, a Mississippi federal district court judge considered Monsanto’s patent infringement suit against Mitchell and Eddie Scruggs for planting unlicensed Roundup Ready seed. The defendants alleged that Monsanto’s ban on saving seed had an anticompetitive effect that violated federal antitrust law. Citing the *McFarling* case, the judge wrote that, “Monsanto’s no replant policy is not subject to challenge under the antitrust laws.”

Legislators Attempt to Abolish Seed Saving Prohibitions

Is it possible to change the laws that justify bans against saving seed? Wes Shoemyer, a member of the Missouri House of Representatives, promoted legislation that would allow farmers to save the seed from patented, genetically engineered crops. Legislators in Iowa, Ohio, and Minnesota have introduced similar seed-saving bills. While Shoemyer acknowledged that state law cannot limit patent rights, he hoped that his efforts would drive the issue to the federal level.

On June 24, 2004, Representative Marcy Kaptur (D-OH) realized this hope by introducing the Seed Availability and Competition Act (H.R. 4693). Her legislation would allow farmers who plant patented seed to retain seed from the harvest for replanting. This benefit would come with a price: farmers who replant saved seed would have to pay into a

Patented Seed Fund. Administered by the U.S. Department of Agriculture, the fund would be used to reimburse the relevant patent holders. This safe harbor for retaining and using seed would override any contractual limitations or obligations to pay royalties or licensing fees.

While the National Farmers Union (<http://www.nfu.org>) and the American Corn Growers Association (<http://www.acga.org>) supported the bill, not all agricultural trade organizations agreed. The National Cotton Council (<http://www.cotton.org>), the National Corn Growers Association (<http://www.ncga.com>) and the American Soybean Association (<http://www.soygrowers.com>) sent a letter to members of Congress expressing their concern about the legislation. The groups asserted that the bill would transform the current marketplace-driven system into a government-administered program and eliminate incentive for companies to invest in long-term research and development required to produce enhanced seed attributes. The USDA has been silent about its enthusiasm for the proposed administrative burden.

The House parked the Seed Availability and Competition Act in two committees throughout 2004. Kaptur promised to reintroduce the bill this year. It is unclear, however, whether the legislation could inspire more enthusiasm this year than last. The Center for Food Safety explores other options for resolving conflicts over saved seed in its report, “Monsanto vs. U.S. Farmers.” Professor Drew L. Kershen offers his analysis of the document in this *ISB News Report*.

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Monsanto vs. U.S. Farmers

Commentary on the Center for Food Safety Report

Drew L. Kershen

In January 2005, the Center for Food Safety (CFS) released a report – MONSANTO vs. U.S. FARMERS – described as



“an extensive review of Monsanto’s use and abuse of U.S. patent law to control the usage of staple crop seeds by U.S. farmers.” As stated in the CFS’s press release accompanying the report, “These law suits and settlements are nothing less than corporate extortion of American farmers. ... suing innocent farmers. We [the CFS] are committed to stopping the corporate persecution of our farmers in its tracks.”

The report consists of five chapters covering fifty-six pages, four pages of endnotes, and an Appendix of seventeen pages in which CFS describes 98 lawsuits against 90 farmer-defendants. These ninety-eight lawsuits serve as the primary, but not sole, data upon which CFS bases its claim of persecution of U.S. farmers.

If one looks at CFS’s own data on the ninety-eight lawsuits in the Appendix, the following results are tabulated. Against the ninety defendants, Monsanto has won seventy-three times. Farmer-Defendants and Monsanto have unresolved lawsuits in fifteen cases in January 2005—so there is, as yet, no winner in these fifteen cases. CFS’s data was unable to determine the outcome of two cases, leaving it unclear whether Monsanto or the farmer-defendant triumphed in the litigation.

When Monsanto wins 73 of 73 cases (of known outcomes), with a number of legal wins coming in front of a jury, it is difficult to agree with CFS that Monsanto is persecuting farmers. Putting aside CFS’s ideological dislike of Monsanto, a dispassionate reader of CFS’s own data would more likely conclude that Monsanto must be pursuing cases only when convinced that it can prove and win the lawsuit. When Monsanto wins 73 of 73 cases, the more accurate description would appear to be that judges and juries concluded that Monsanto was protecting its legal rights against defendants who had factually infringed those legal rights.

CFS also claims that Monsanto is “suing innocent farmers.” Monsanto’s 73 wins in 73 cases seems difficult to reconcile with CFS’s claim of “suing innocent farmers.” Moreover, in the judicial opinions that courts have published about these 98 lawsuits, all the farmers, except one, have admitted that they intentionally acquired Monsanto patented seed without signing a license agreement or that they purposefully saved Monsanto patented seed in violation of the signed technology use agreement prohibiting the saving of seed for replanting in the following year. By deciding for Monsanto, judges and juries have obviously indicated difficulty in applying the label “innocent” to these farmer-defendants.

When one looks at CFS’s data, it becomes clear that what CFS finds unacceptable is that the statutory law, the judges,

and the juries favor Monsanto. In other words, while CFS vents its rhetorical wrath on Monsanto, CFS actually is complaining about the law and its effective enforcement. CFS’s complaint becomes clear when one reads the report’s Chapter 5 “Policy Options: Preventing the Prosecution of America’s Farmers.”

- Amend the Patent Act (PA) and the Plant Variety Protection Act (PVPA) to exclude plants from the subject matter that can be protected by intellectual property rights;
- If the PA and the PVPA are not amended to exclude plants, make the PVPA the exclusive statutory means for protecting plants as intellectual property because the PVPA has an exception for farmers saving seeds for replanting on their own lands;
- Amend the PA to add a farmers’ saved-seed exception and /or to exempt inadvertent possession from being an infringing act;
- Legislate liability laws to put liability on seed companies (Monsanto);
- Adopt existing state models for controlling the intrusive and aggressive patent infringement investigations of farmers;
- Pass legislation that negates the forum selection clause in technology use agreements;
- Pass statutes or ordinances banning the growing of transgenic crops.

Of these seven policy options, bullet points 1, 2, 3, 5 and 6 are calls for amendments or new laws that prevent or reduce enforcement of intellectual property rights. Bullet points 4 and 7 have nothing to do with the report’s study of the “use and abuse” of US patent laws but are direct attacks on agricultural biotechnology. Indeed, the CFS report was released in January 2005 just as legislators in Vermont, Montana, North Dakota, and Hawaii introduced CFS-drafted legislation about liability that embodied the CFS policy option bullet point 4.

After reading the CFS report, the author is reminded of Sinclair Lewis’ lament about the public reaction to his novel, *The Jungle*, “I aimed at the public’s heart and by accident I hit it in the stomach.” The CFS report claims to aim at farmers’ innocent hearts and persecuted souls but actually hits guilty farmers’ in their back (wallet) pockets. Other farmers, who by the hundreds of thousands have used transgenic seeds without conflict with Monsanto, may not be impressed with CFS’s aim.

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