Stepping Back

How can we improve regulatory reviews to promote innovative and safe uses of genetically modified trees?

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Goals for today

- Rationale for change
  - Urgent need and context for genetic innovation
  - Impressive record of research accomplishment from field studies of GE trees
  - Severe constraints to research and breeding from preclusion of gene flow
- Regulatory revisions
  - Exemptions of the familiar and similar
  - Tolerances for gene flow and management
- Stepping back

Billions are struggling now, and it's a very scary future – agriculture and forestry of all kinds will become <u>much</u> more difficult

No-analog thinking

PALEOECOLOGY PALEOECOLOGY PALEOECOLOGY

475

Novel climates, no-analog communities, and ecological surprises

John W Williams[1*] and Stephen T Jackson[2]

No-analog communities (communities that are compositionally unlike any found today) occurred frequently in the past and will develop in the greenhouse world of the future. The well documented no-analog plant communities of late-glacial North America are closely linked to "novel" climates also lacking modern analogs, characterized by high seasonality of temperature. In climate simulations for the Intergovernmental Panel on Climate Change A2 and B1 emission scenarios, novel climates arise by 2100 AD, primarily in tropical and subtropical regions. These future novel climates are warmer than any present climates globally, with spatially variable shifts in precipitation, and increase the risk of species reshuffling into future no-analog communities and other ecological surprises. Most ecological models are at least partially parameterized from modern observations and so may fail to accurately predict ecological responses to these novel climates. There is an urgent need to test the robustness of ecological models to climate conditions outside modern experience.

Front Ecol Environ 2007; 5(9): 475–482, doi:10.1890/070037

How do you study an ecosystem no ecologist has ever seen? This is a problem for both paleoecologists and global-change ecologists, who seek to understand ecolog- … past or future, is heavily conditioned by our current observations and personal experience.

The further our explorations carry us from the present …

"No-analog communities (communities that are compositionally unlike any found today) occurred frequently in the past and will develop in the greenhouse world of the future."

Constraints to breeding with trees are great – GE methods offer very significant additional tools

Constraints include

- Difficulty to inbreed / introgress new genes
- Long breeding cycle
- Common use of asexually propagated varieties of high value

GE proven to be of diverse value for forest trees

All demonstrated in the field

- Resistance to insects and diseases
- Tolerance to salinity and temperature stress
- Phytoremediation of environmental toxins
- Modified properties to improve processing for biofuels or pulp
- Tolerance to herbicides to reduce the environmental impacts, improve efficiency, or reduce costs of weed control treatments

GE proven to be of diverse value for trees

All demonstrated in the field

- Accelerated flowering for faster breeding and research
- Fertility control for reduced spread and improved growth rate
- Synthesis of new, renewable bioproducts

Yet there is hardly a trickle of commercial GE tree products compared to its scientific potential – why?

Social / market and regulatory barriers are great

Global admixture of GM and non-GM crops/food create immense coexistence, trade problems under current regulations

Many costly cases of trade disruption and lawsuits with corn, soy, and rice – billions in lost value

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Steady increase in incidents of genetically modified crops found in traded food, UN agency reports

Source: UN Photo/Tobin Jones

14 March 2014 – As a result of the increased production of genetically modified crops worldwide, the United Nations food agency warns in a ground-breaking survey that an increasing number of incidents of low levels of genetically modified organisms (GMOs) are being reported in traded food and feed.

18 Like

http://www.un.org/apps/news/story.asp?NewsID=47354&Cr=food+security&Cr1=#.UySzoPldVUV

Oregon GMO “wheat-gate” shows the huge risk in doing research

OREGON'S ACCIDENTAL CROP

The discovery of GE wheat highlights regulatory failures

BRENT WOJAHN/THE OREGONIAN

Wheat grows near Condon in north-central Oregon. Rogue genetically modified wheat found in an eastern Oregon field has prompted debate over how to contain, and how the U.S. regulates, genetically engineered crops.

SSANEIN

ineered wheat g in an eastern

trials, ensure that performance standards are met and trace back the source of contamination that might occur as a result of GE experiments. This lack of basic information not only hampers the government, but also threatens the agricultural

Op-Ed in Oregonian
June 16, 2013

An agreed safe, well studied, extremely rare GMO left over from earlier research nearly crippled Pacific Northwest trade in wheat, led to lawsuits

The problem much worse for most trees

Molecular Ecology (2009) **18**, 357–373 doi: 10.1111/j.1365-294X.2008.04016.x

Extensive pollen flow in two ecologically contrasting populations of *Populus trichocarpa*

G. T. SLAVOV,*† S. LEONARDI,‡ J. BURCZYK,§ W. T. ADAMS,¶ S. H. STRAUSS¶ and S. P. DIFAZIO*

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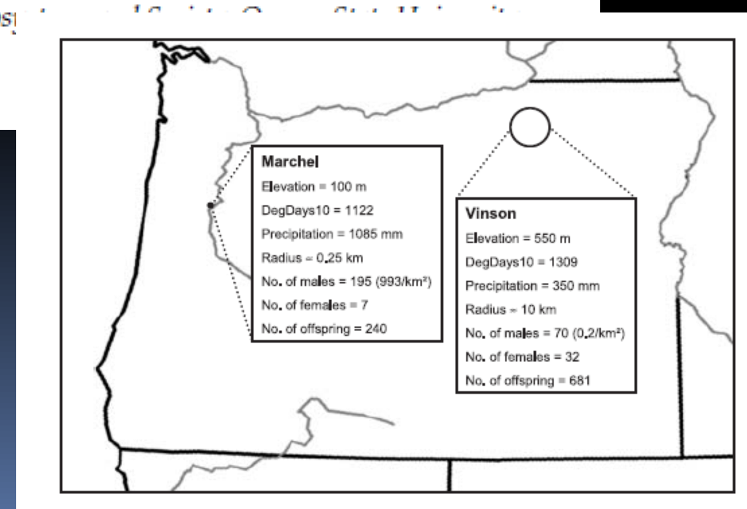
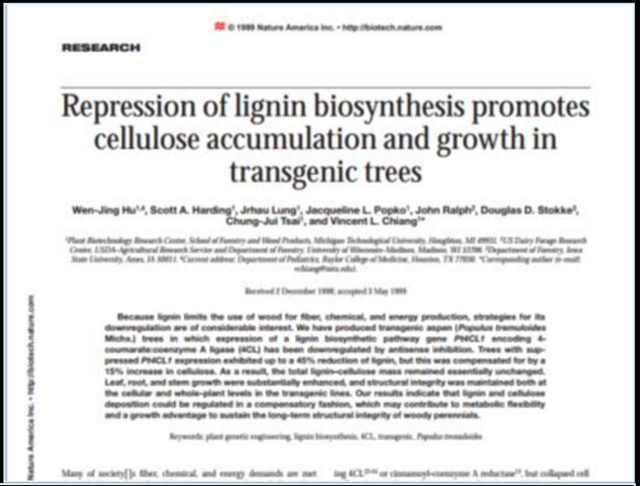


Field studies essential for complex trits

The case of the magic lignin-reduced trees

- Nature Biotechnology 1999 – antisense *4CL* genes generated much excitement
- Increase of growth rate, halving of lignin content, no obvious ill effects in greenhouse



RESEARCH

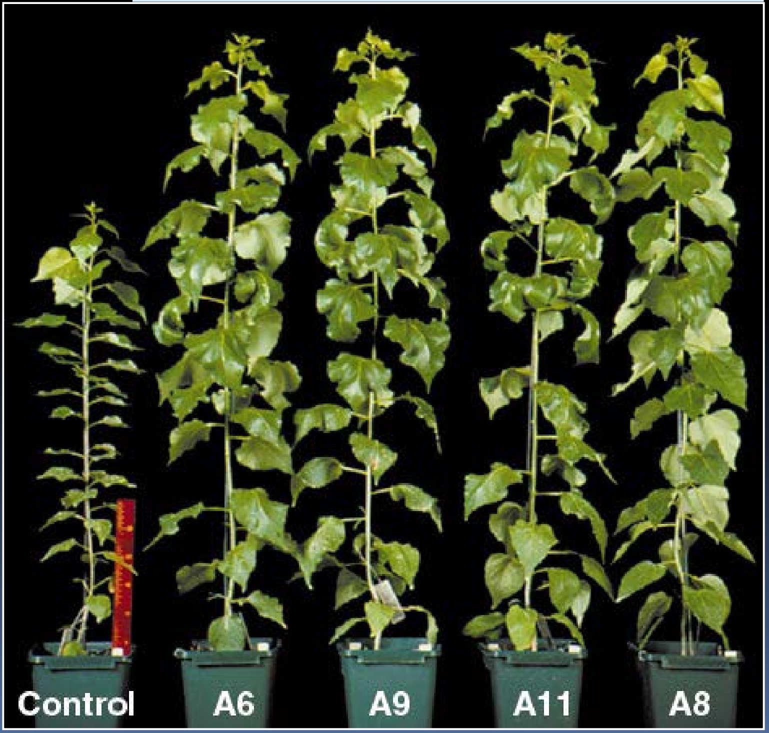
Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees

Wen-Jing Hu[1,4], Scott A. Harding[1], Jrhau Lung[1], Jacqueline L. Popko[1], John Ralph[2], Douglas D. Stokke[3], Chung-Jui Tsai[1], and Vincent L. Chiang[1*]

Received 2 December 1998; accepted 3 May 1999

Because lignin limits the use of wood for fiber, chemical, and energy production, strategies for its downregulation are of considerable interest. We have produced transgenic aspen (*Populus tremuloides* Michx.) trees in which expression of a lignin biosynthetic pathway gene *Pt4CL1* encoding 4-coumarate:coenzyme A ligase (4CL) has been downregulated by antisense inhibition. Trees with suppressed *Pt4CL1* expression exhibited up to a 45% reduction of lignin, but this was compensated for by a 15% increase in cellulose. As a result, the total lignin–cellulose mass remained essentially unchanged. Leaf, root, and stem growth were substantially enhanced, and structural integrity was maintained both at the cellular and whole-plant levels in the transgenic lines. Our results indicate that lignin and cellulose deposition could be regulated in a compensatory fashion, which may contribute to metabolic flexibility and a growth advantage to sustain the long-term structural integrity of woody perennials.

Keywords: plant genetic engineering, lignin biosynthesis, 4CL, transgenic, *Populus tremuloides*



Its totally different in the field

Research

New Phytologist

Reduced wood stiffness and strength, and altered stem form, in young antisense *4CL* transgenic poplars with reduced lignin contents

Steven L. Voelker[1], Barbara Lachenbruch[1], Frederick C. Meinzer[2] and Steven H. Strauss[3]

[1]Department of Wood Science & Engineering, Oregon State University, Corvallis, OR 97330, USA; [2]USDA Forest Service, Pacific Northwest Research Station, 3200 Jefferson Way, Corvallis, OR 97330, USA; [3]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97330, USA

Plant, Cell & Environment

PC&E

Plant, Cell and Environment (2011) doi: 10.1111/j.1365-3040.2010.02270.x

Transgenic poplars with reduced lignin show impaired xylem conductivity, growth efficiency and survival

STEVEN L. VOELKER[1], BARBARA LACHENBRUCH[1], FREDERICK C. MEINZER[2], PETER KITIN[3] & STEVEN H. STRAUSS[4]

[1]Department of Wood Science and Engineering and [4]Department of Forest Ecosystems and Society, Oregon State University,
[2]U.S.D.A. Forest Service, Forest Sciences Laboratory, 3
Wood Biology and Xylarium, Royal Museum for Cent

Antisense Down-Regulation of *4CL* Expression Alters Lignification, Tree Growth, and Saccharification Potential of Field-Grown Poplar[1[W][OA]]

Steven L. Voelker, Barbara Lachenbruch, Frederick C. Meinzer, Michael Jourdes, Chanyoung Ki, Ann M. Patten, Laurence B. Davin, Norman G. Lewis, Gerald A. Tuskan, Lee Gunter, Stephen R. Decker, Michael J. Selig, Robert Sykes, Michael E. Himmel, Peter Kitin, Olga Shevchenko, and Steven H. Strauss*

Department of Wood Science and Engineering (S.L.V., B.L.) and Department of Forest Ecosystems and Society (O.S., S.H.S.), Oregon State University, Corvallis, Oregon 97331; United States Department of Agriculture Forest Service, Pacific Northwest Research Station, Corvallis, Oregon 97331 (F.C.M.); Washington State University, Institute of Biological Chemistry, Pullman, Washington 99164–6340 (M.J., C.K., A.M.P., L.B.D., N.G.L.); BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831–6422 (G.A.T., L.G., S.R.D., M.J.S., R.S., M.E.H.); National Rene
L.G., S.R.D., M.J.S., R.S., M.E.H.); and Laborator
Central Africa, B–3080 Tervuren, Belgium (P.K.)

Plant Physiology, October 2010

The core problem: Presumption of harm from GE method during research and breeding

- All gene flow must be prevented during research
  - But movement from mature trees will occur due to incomplete domestication, wild and feral relatives, wide pollen and often seed movement
- Impedes or prevents stress resistance and other complex trait development
  - Require extensive field trials, through to tree maturity, to test many concepts and insertion events
- Increasingly an anachronism in the era of precision breeding, cisgenics, intragenics

An additional issue: Event-specific decisions and costs

- Slowness/difficulty of introgression – essentially unused in forestry
- Need diverse genes and genotypes transformed <u>during</u> breeding program
- Small economic benefits to pay back regulatory costs from single events
- Gene flow and AP/LLP a nightmare during research and breeding with many genes, genotypes, and events

A serious regulatory problem under USA system

Articles

Far-reaching Deleterious Impacts of Regulations on Research and Environmental Studies of Recombinant DNA-modified Perennial Biofuel Crops in the United States

STEVEN H. STRAUSS, DREW L. KERSHEN, JOE H. BOUTON, THOMAS P. REDICK, HUIMIN TAN, AND ROGER A. SEDJO

October 2010 / Vol. 60 No. 9 • BioScience 729

International regulatory pressure in wrong direction due to Cartagena Pr.

Strangled at birth? Forest biotech and the Convention on Biological Diversity

Steven H Strauss, Huimin Tan, Wout Boerjan & Roger Sedjo

Against the Cartagena Protocol and widespread scientific support for a case-by-case approach to regulation, the Convention on Biological Diversity has become a platform for imposing broad restrictions on research and development of all types of transgenic trees.

The Convention on Biological Diversity (CBD) has become a major focus of activist groups that wish to ban field research and commercial development of all types of genetically modified (GM) trees. Recent efforts to influence CBD recommendations by such groups has led to the adoption of recommendations for increased regulatory stringency that are inconsistent with the views of most scientists and most of the major environmental organizations. We suggest that the increasingly stringent recommendations adopted by the CBD in recent years are impeding, and in many places may foreclose, much of the field research needed to develop useful and safe applications of

A convention co-opted

Negotiated under the United Nations (UN) Environment Program, CBD was adopted in June 1992 and subsequently entered into force in December 1993. The CBD has been signed by 191 of the 192 members of the UN, making it one of the largest international treaties. The aim of the CBD is to promote the conservation and sustainable use of biodiversity, and the fair and equitable sharing of benefits from the use of genetic resources. Because transgenic organisms have the potential to affect biodiversity, special provisions of the CBD cover the use and trade in living modified organisms (LMOs, also known as genetically modified organisms; GMOs).

In 2000, the Cartagena Protocol on Biosafety ... the CBD

An example of the perverse risks of method-based regulation: “Catkin-gate”

The strange case of the upright summer catkin


802
© AP Photo/Rogelio V. Solis

Regulatory confusion, obstacles at national and international levels

Feature

The Phantom Forest: Research on Gene-Altered Trees Leaps Ahead, into a Regulatory Limbo

STEVE NASH

At an industrial park in Walnut Creek, California, technicians and robots are sorting through the 550 million base pairs of genetic code in poplar DNA to sequence a tree genome for the first time. They are poised to unlock a fine, full toolbox for the work of genetic engineering in trees.

In Vermont, a group called Action for Social and Ecological Justice has just kicked off a national campaign to pressure companies to ban research on genetically engineered (GE) trees. The Sierra Club, the World Wildlife Fund, and the American Lands Alliance, among others, have called for a moratorium on commercialization of GE trees.

In Washington, a federal agency with key responsibility for judging the biological safety of GE trees is preparing its response for Congress to a report by the

More than 200 notices of field trials have been filed with federal regulators for lab-engineered fruit, nut, and forest trees—also known as genetically modified, biotech, or transgenic trees. But aside from a virus-resistant, bushlike papaya tree grown in Hawaii, no one has yet sought regulatory approval for commercial use of a gene-altered tree.

"Maybe soon,

codirector of the

group at North C

Like others in th

feels little certain

Westvaco Corporation, and two New Zealand firms. Arborgen estimates that, if tests go very well, the product could be ready for the market in a decade.

Cloned cathedrals

Tinkering with tree DNA presents some issues for research and for public policy, however. Casting an uncertain light over

Lignin-modified trees

Concept proven, but much refinement needed

Type of gene, promoters, extent of modification, environment, stand management, genotype modified

PNAS PNAS PNAS

Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase

Rebecca Van Acker[a,b], Jean-Charles Leplé[c], Dirk Aerts[d], Véronique Storme[a,b], Geert Goeminne[a,b], Bart Ivens[a,b], Frédéric Légée[e], Catherine Lapierre[e], Kathleen Piens[f], Marc C. E. Van Montagu[a,b,1], Nicholas Santoro[g], Clifton E. Foster[g], John Ralph[h], Wim Soetaert[d], Gilles Pilate[c], and Wout Boerjan[a,b,1]

[a]Department of Plant Systems Biology, VIB, 9052 Ghent, Belgium; [b]Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium; [c]Institut National de la Recherche Agronomique (INRA), Unité de Recherche 0588, Amélioration, Génétique et Physiologie Forestières, 45075 Orléans, France; [d]Centre of Expertise for Industrial Biotechnology and Biocatalysis, Ghent University, 9000 Ghent, Belgium; [e]Institut Jean-Pierre Bourgin, Unité Mixte de Recherche 1318, INRA-AgroParisTech, INRA Centre de Versailles-Grignon, 78026 Versailles, France; [f]Department of Biochemistry and Microbiology, Ghent University, 9000 Ghent, Belgium; [g]Department of Energy Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI 48824; and [h]Departments of Biochemistry and Biological Systems Engineering, Wisconsin Energy Institute, and the Department of Energy Great Lakes Bioenergy Research Center, University of Wisconsin–Madison, Madison, WI 53726

Contributed by Marc C. E. Van Montagu, November 20, 2013 (sent for review March 26, 2013)

Lignin is one of the main factors determining recalcitrance to enzymatic processing of lignocellulosic biomass. Poplars (*Populus tremula* x *Populus alba*) down-regulated for cinnamoyl-CoA reductase (CCR), the enzyme catalyzing the first step in the monolignol-specific branch of the lignin biosynthetic pathway, were grown in field trials in Belgium and France under short-rotation coppice culture. Wood samples were classified according to the intensity of the red xylem coloration typically associated with *CCR* down-regulation. Saccharification assays under different pretreatment conditions (none, two alkaline, and one acid pretreatment) and simultaneous saccharification and fermentation assays showed that wood from the most affected transgenic trees had up to 161% increased ethanol yield. Fermentations of combined material from the complete set of 20-mo-old *CCR*–down-regulated trees, including bark and less efficiently down-regulated trees, still yielded ~20% more ethanol on a weight basis. However, strong down-regulation of *CCR* also affected biomass yield. We conclude that *CCR* down-regulation may become a successful strategy to improve biomass processing if the variability in down-regulation and the yield penalty can be overcome.

bioethanol | GM | second-generation bioenergy

Global warming and the depletion of fossil fuels provide a major impetus for the increased interest in renewable energy sources. Liquid biofuels, bioethanol in particular, are cur-

incorporated into the lignin polymer, respectively (5–7). Cinnamoyl-CoA reductase (CCR) catalyzes the first step of the monolignol-specific pathway. It converts the hydroxycinnamoyl-CoA esters to their corresponding hydroxycinnamaldehydes (mainly feruloyl-CoA to coniferaldehyde), and down-regulation of *CCR* typically results in reduced lignin content (8–13). *CCR*–down-regulated poplars are characterized by an orange to wine-red coloration of the xylem that often appears in patches along the stem. This pronounced coloration is associated with a reduction in lignin amount and the incorporation of low levels of ferulic acid into the polymer (13, 14).

As lignin is the most important factor limiting the conversion of plant biomass to fermentable sugars (15–17), we have evaluated whether wood from transgenic poplar, down-regulated in *CCR*, is easier to process into ethanol. Field trials were established in Belgium and France after a long process of obtaining regulatory permission (18). Field trials are an essential step in translating fundamental knowledge generated in the laboratory to conditions closer to industrial exploitation because greenhouse-derived data cannot a priori be extrapolated to field-grown trees without experimentation. For example, greenhouse-grown trees do not experience the annual cycles of growth and

Significance

In the transition from a fossil-based to a bio-based economy, bioethanol will be generated from the lignocellulosic biomass

PLANT BIOLOGY

Cold tolerant *Eucalyptus*

Concept proven, much refinement needed

Type of gene, promoters, extent of modification, environment, stand age, genotype modified

Provided by Arborgen

Forest pest epidemics increasing with travel and climate change

Regulations make timely use impossible

Examples

1892 - White pine blister rust

1904 - Chestnut blight

1923 - Port-Orford-cedar root disease

1920s - Beech scale complex

1930 - Dutch elm disease

1967 - Butternut canker

1976 - Dogwood anthracnose

2000s - Sudden oak death

American elm

Goals for today

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Proposed regulatory solutions – tiered regulation, product vs. process

POLICY FORUM

GENETIC TECHNOLOGIES

Genomics, Genetic Engineering, and Domestication of Crops

Steven H. Strauss

Genomic sequencing projects are rapidly revealing the content and organization of crop genomes (*1*). By isolating a gene from its background and deliberately modifying its expression, genetic engineering allows the impacts of all genes on their biochemical networks and organismal phenotypes to be discerned, regardless of their level of natural polymorphism. This greatly increases the ability to determine gene function and, thus, to identify new options for crop domestication (*2*). The organismal functions of the large majority of genes in genomic databases are unknown.

portant to agricultural goals, but poorly represented in breeding populations because they are rare or deleterious to wild progeni-

huge numerical obstacle that is normally provided by extant wild and domesticated gene pools. Despite the great diversity of genes that can comprise GGTs, many of the modified traits are familiar, having a long history of domestication and consequent reduced fitness through artificial selection. Male sterility, seedless fruits, delayed spoilage, and dwarf stature are familiar examples.

GGTs that improve abiotic stress tolerance

Confinement level	Type 1 field trials (exploratory)	Type 2 field trials (precommercial)	Examples
High	Biological and physical confinement—detailed data		Highly toxic or allergenic pharmaceuticals and proteins
Medium	FSC, basic data	FSC, detailed data	Novel pest-management genes, low toxicity pharmaceuticals and proteins
Low: Stress tolerance	FSC, basic data	FSC, detailed data	Genomics-guided transgenes
Low: Domesticating	Petition for exemption?	FSC, basic data	

Categories of confinement and monitoring for small- and large-scale transgenic field trials. Biological confinement includes genetic mechanisms to preclude spread and/or reproduction. Physical confinement requires use of geographical isolation or physical barriers. FSC, farm-scale confinement; use of spatial isolation within and between farms and border crops, combined with ...oring. Detailed data include surveys of gene flow away from the site. Basic data ...hment of confinement mechanisms.

www.sciencemag.org SCIENCE VOL 300 4 APRIL 2003

PERSPECTIVE

nature biotechnology

Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics

Kent J Bradford[1], Allen Van Deynze[1], Neal Gutterson[2], Wayne Parrott[3] & Steven H Strauss[4]

The costs of meeting regulatory requirements and market restrictions guided by regulatory criteria are substantial impediments to the commercialization of transgenic crops. Although a cautious approach may have been prudent initially, we argue that some regulatory requirements can now be modified to reduce costs and uncertainty without compromising safety. Long-accepted plant breeding methods for incorporating new diversity into crop varieties, experience from two decades

Regulatory costs also play a role in the growing disparity between the expanding global adoption of the large-market transgenic maize, soybean, cotton and canola crops[1] and the so-called 'small-market' or 'specialty' crops, for which field trials and commercial releases of transgenic food crops have all but stopped[3]. In 2003, fruits, vegetables, landscape plants and ornamental crops accounted for more than $50 billion in value in the United States, representing 47% of the total US farm crop income[9]. Of this, the only transgenic commodities currently mar-

Gene targeting, genome editing, coming along fast

= increased precision, safer than breeding

NEWS & VIEWS

NATURE|Vol 459|21 May 2009

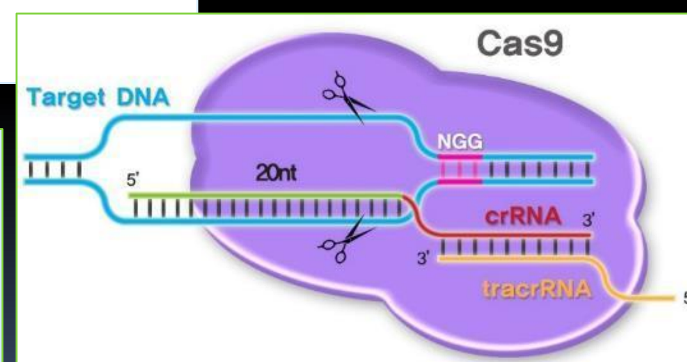
PLANT BIOTECHNOLOGY

Zinc fingers on target

Matthew H. Porteus

The existing methods of creating genetically modified plants are inefficient and imprecise. Zinc-finger technology offers the prospect of opening up a swifter and more exact route for crop improvement.

CRISPRS



TALENs

Suggested exemptions – a start

- Approved, familiar markers and gene transfer systems based on approvals in other crops
- Mutagenesis of transformation system
- Cisgenic (or functionally cisgenic) transfers from similar or closely related species (e.g., congeneric gene sources)
- Modification of expression of native genes and pathways (intragenic)
- Genome editing or mutagenesis

Suggested exemptions – a start

- Well understood products with urgent ecological or humanitarian value, and non-toxic
  - USA: Early consult with FDA re. low level admixture
- Gene dispersal into the environment and associated AP/LLP during research and breeding, or when crop-appropriate mitigation methods are employed
- Best management practices (BMPs) not zero-tolerance

Exemptions and lower tiers of regulation do not mean all GMOs unregulated

- Companies to choose regulatory reviews where desired, or with high novelty or risk
- Right of agencies to challenge based on trait novelty and scientific reviews
- Food safety, environmental benefit vs. hazard, trade hazards beyond newly set AP thresholds
- Presumptive value of innovation and safety, vs. presumption of harm due to method
  - Comparator is conventional breeding and plant domestication practices

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In summary

- Growing population, living standards, and climate change pose existential challenges to civilization, economics, and livelihoods everywhere
- Ecosystems in the near future (one or a few tree generations) will change radically
- Breeding and genetics are not panaceas, but are powerful tools to help manage these threats

In summary

- GE has proven itself a very powerful new genetic tool for both crops and trees
- Demand precaution, not the precautionary principle
  - We need all major tools if we are to be able to cope with a frightening future
- Develop and use GE methods based on product familiarity, benefits, and safety
  - Not based on the method or unworkable method-based AP/LLP rules

Voltaire was right….

The perfect

is the enemy

of the good