"Genetic Engineering New Crops: Importance for Food, Fuel, and Sustainable Crops"

Bob Goldberg
9/23/08
Today's Headlines

The New York Times

A Global Need for Grain That Farms Can’t Fill
Published: March 9, 2008

Los Angeles Times

Economist.com

High Rice Cost Creating Fears of Asia Unrest
By KEITH BRADSHER
Published: March 29, 2008

U.S. News

CNN.com

A Drought in Australia, a Global Shortage of Rice

Across Globe, Empty Bellies Bring Rising Anger

Newsweek

Riots, Instability Spread as Food Prices Skyrocket
We Face Challenges In Agriculture Even Greater Than Those in Today’s Headlines

Over the next 50 years we will need to produce more food than in the whole of human history and do it with fewer inputs on less arable land!!!!

Crop yields need to be increased significantly!!
Without Increases in Crop Yield We Will Need to Farm Every “Square Inch” of Land on the Earth To Satisfy Crop Demand

There is a Limited Amount of Land For Agriculture

NOTE:
More Yield per Acre Leaves More Land for Nature!!!!!
Plants Require Water and Nutrients (e.g., Nitrogen) to Grow!!

More Than 90% of the World’s Land is Unsuitable for Growing Crops

Pests (insects, fungi, bacteria, & viruses) Make Farming Even More Difficult!!
And Foods Unsafe (e.g., mycotoxins)!!

Growing Crops in Harsh Environments is not “Natural!!”
And........There's Also A Problem With Using Land For Energy Production..............
There Were 18,000 Farms in Los Angeles County in 1930!!!
From 1901 to 1950 Los Angeles County Was the Largest Agricultural Producing County in the US!!!

Today's Lecture

Original Agricultural College and Citrus/Avocado Orchard

Thelmer Hoover
4/11/29
How Will Crop Yields Be Increased?

As We Always Have...........
By Using State-of-the-Art Science & Technology
And By Using a Variety of Approaches to Identify Genes and Processes That Will Help Increase Crop Yields and Food Production Significantly in the 21st Century

**Yield (Developmental Traits)**
- Seed Number
- Seed Size
- Growth Rate
- Organ Size *(More Seeds)*
- Plant Architecture
- Flowering Time
- Senescence
- Maturity
- Stature

**Yield (Stress Traits)**
- Nutrient Uptake
- Drought Resistance
- Heat Resistance
- Cold Tolerance
- Salt Tolerance
- Shade Tolerance
- Disease Resistance

From “Low-Tech” to High-Tech

From Lab to Improved Seeds For Farmers
And Use Breeding and Genetic Engineering to Introduce These “Yield” Genes Into Existing Crops

This WILL Happen......Sooner Than Later!
In the Beginning....

Seed
Early Humans Faced Major Challenges Finding Food

Inventing Agriculture and “Domesticating” Plants and Animals 10,000 Years Ago Changed That & Everything Else!!!
The Invention of Agriculture Led to Civilization As We Know It!!!

Agriculture Dates Back 10,000 Years

Agriculture Dates Back 10,000 Years
Generating New Types Of Crops Is Not New To The 21st Century!!

Crops of Egypt - 400 BC
All Major Food Crops Were “Engineered” By Breeding ~10,000 Years Ago

- **SEEDS (cereals):** corn, rice, wheat, barley, millet, sorghum
- **SEEDS (legumes):** soybean, beans, peanut
- **ROOTS AND STEMS:** potato, cassava, yam, sugar beet, sugar cane, radish
- **FRUITS:** tomato, banana, coconut, papaya
- **LEAVES:** cabbage, kale, lettuce, spinach
- **FLOWERS:** broccoli, cauliflower, artichoke

Crops were selected by using pre-existing genetic variability in wild plant populations -- They Were Made by “Man” and Not by Nature!!

*Breeding, By Definition, Means Manipulating Genes!!!!*
Engineering Teosinte Into Domesticated Corn

Note: Architecture and Fruit (cob) Size
Modern Corn Was “Engineered” From Teosinte 10,000 Years Ago & Cannot Survive in “Nature!!”
Tomatoes Were Engineered From Small Wild Relatives
Broccoli, Cauliflower, Cabbage, and Brussels Sprouts Were “Engineered” As Well!

......Brassicas or Crucifers
Big Changes in the US Over The Past 100 Years
“We’ve Come a Long Way Baby”

<table>
<thead>
<tr>
<th></th>
<th>1900</th>
<th>2008</th>
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<tbody>
<tr>
<td>Life Expectancy</td>
<td>48 (women)</td>
<td>79 (women)</td>
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<tr>
<td>Average Family Income</td>
<td>$8,000</td>
<td>$50,000</td>
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<tr>
<td>(2008 Dollars)</td>
<td></td>
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</tr>
<tr>
<td>Gasoline Use Per Capita</td>
<td>34 gallons</td>
<td>1,100 gallons</td>
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<tr>
<td>Flush Toilets Per Housing</td>
<td>10%</td>
<td>99%</td>
</tr>
<tr>
<td>High School Grads</td>
<td>13%</td>
<td>90%</td>
</tr>
<tr>
<td>Farm Workers</td>
<td>55%</td>
<td>1.5%</td>
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CROP YIELD INCREASES HAVE “ROCKETED UPWARDS” OVER THE LAST 100 YEARS AND CONTRIBUTED TO A LONGER AND “BETTER” LIFE

<table>
<thead>
<tr>
<th>% Farm Workers</th>
<th>% Income on Food</th>
<th>Life Span</th>
</tr>
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<tbody>
<tr>
<td>55%</td>
<td>50%</td>
<td>48 Years</td>
</tr>
<tr>
<td>1.5%</td>
<td>9%</td>
<td>79 Years</td>
</tr>
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<table>
<thead>
<tr>
<th>Year</th>
<th>Crop Yield (bushels/acre)</th>
<th>Farmers/Food Cost</th>
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<tbody>
<tr>
<td>1900</td>
<td>100</td>
<td>1900: 30 bushels/acre</td>
</tr>
<tr>
<td>1920</td>
<td>115</td>
<td>2008: 150 bushels/acre</td>
</tr>
<tr>
<td>1940</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>1950</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>300</td>
<td></td>
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</tbody>
</table>

Conclusion: Crop yield increased ~ 300% over the past 100 years and lead to a similar reduction in food costs!!!!!
How Was This Accomplished Over the Past 100 Years?

What Role Did Science & Technology Play?

What About in the Future When There are 400 Million People in the USA and 9 Billion in the World?
WHAT TECHNOLOGIES CAUSED AN INCREASE IN CROP YIELDS OVER THE PAST 100 YEARS?

• PLANT BREEDING (New Hybrids-Green Revolution)
• IRRIGATION
• FERTILIZERS
• PESTICIDES & HERBICIDES
• MECHANIZATION (e.g., Tractor)
• GLOBAL POSITIONING AND SATELLITE IMAGING
• GENOMICS & GENETIC ENGINEERING (New Traits)

These technologies have resulted in a 300% increase in US crop productivity during the 20th-21st century! Need to sustain this yield increase by applying the best technology and agricultural practices!
1900: Rediscovery of Mendel’s Work

DeVries, Correns and Tschermak independently rediscovered Mendel’s work.

Three botanists - Hugo DeVries, Carl Correns and Erich von Tschermak - independently rediscovered Mendel’s work in the same year, a generation after Mendel published his papers. They helped expand awareness of the Mendelian laws of inheritance in the scientific world.

The three Europeans, unknown to each other, were working on different plant hybrids when they each worked out the laws of inheritance. When they reviewed the literature before publishing their own results, they were startled to find Mendel’s old papers spelling out those laws in detail. Each man announced Mendel’s discoveries and his own work as confirmation of them.

1909: The Word Gene Coined

Danish botanist Wilhelm Johannsen coined the word gene to describe the Mendelian units of heredity.

He also made the distinction between the outward appearance of an individual (phenotype) and its genetic traits (genotype).

Four years earlier, William Bateson, an early geneticist and a proponent of Mendel’s ideas, had used the word genetics in a letter; he felt the need for a new term to describe the study of heredity and inherited variations. But the term didn’t start spreading until Wilhelm Johannsen suggested that the Mendelian factors of inheritance be called genes.

The proposed word traced from the Greek word genos, meaning “birth”. The word spawned others, like genome.

1911: Fruit Flies Illuminate the Chromosome Theory

Using fruit flies as a model organism, Thomas Hunt Morgan and his group at Columbia University showed that genes, strung on chromosomes, are the units of heredity.

Morgan and his students made many important contributions to genetics. His students, who included such important geneticists as Alfred Sturtevant, Hermann Muller and Calvin Bridges, studied the fruit fly Drosophila melanogaster. They showed that chromosomes carry genes, discovered genetic linkage - the fact that genes are arrayed on linear chromosomes - and described chromosome recombination.

In 1933, Morgan received the Nobel Prize in Physiology or Medicine for helping establish the chromosome theory of inheritance.

2000: Drosophila and Arabidopsis genomes sequenced

Drosophila melanogaster (fruit fly) has been a primary tool for geneticists since the early part of the 20th century. The sequencing of its genome is the result of a collaborative effort between the Drosophila Genome Project Group, led by Gerald Fink at the University of California, Berkeley and researchers from Osaka University. The Drosophila genome is estimated to have approximately 13,600 genes as compared to 20,000–25,000 genes in humans. The simplicity of Drosophila as an experimental organism means that its genome sequence has been useful in studying human disease. Many of the genes in Drosophila have been conserved through evolution and have human counterparts. This means that scientists can perform experiments using flies and apply their findings to human biology.

Arabidopsis thaliana is the first plant to have its genome sequenced. This plant from the mustard family has become the plant biologist’s equivalent of the laboratory mouse. Its genome was completed by the collective efforts of an international group of researchers called the Arabidopsis Genome Initiative. The Arabidopsis genome has an estimated 25,000 genes—apparently even more than humans. Although not a crop plant, Arabidopsis was chosen as a model organism because its genome is small and it has relatively little of the noncoding, so-called junk DNA. It does, however, share very similar biochemistry to crop plants such as rice or barley. The study of its sequence is expected to have widespread applications for agriculture and medicine.

2004: Refined Analysis of Complete Human Genome Sequence

The International Human Gene Sequencing Consortium led in the United States by the National Human Genome Research Institute and the Department of Energy published a description of the finished human gene sequence. The analysis reduced the estimated number of genes (which is currently the mid-1990s had been ~100,000) from 35,000 to 26,000-35,000. The fact that the human genome has far fewer genes than was originally thought suggests that humans "get more" out of their genetic information than do other animals. For example, the average human gene is able to produce three different gene products.

The finished sequence contains 2.85 billion nucleotides interrupted by only 314 gaps. It covers 99 percent of the genome with an accuracy of 1 error per 100,000 bases. Researchers confirmed the existence of 25,599 protein-coding genes and identified 2,196 other DNA segments that are thought to be protein-coding genes. Although the genome sequence is described as "finished," it isn’t perfect. The small gaps that remain cannot be sequenced by the industrial-scale methods used by the Human Genome Project. Filling in these gaps will have to await a series of small targeted efforts by researchers using other techniques and possibly new technologies. The finished genome sequence can be freely accessed through public databases and may be used by researchers without restrictions.
Modern Genetic Engineering Has Come a Long Way Since Its Origins in 1973!

Gene Transplants Seen Helping Farmers and Doctors;

By VICTOR K. McELHENY
May 20, 1974, Monday
Page 61, 1335 words

Biochemists working in California have developed a practical method of transplanting genes, the chemical units of heredity, from cells as complex as those of animals into the extremely simple, fast-multiplying cells known as bacteria. [END OF FIRST PARAGRAPH]
Unity in Biology

Genetic Engineering

Translating the Genetic Code Into Proteins is a Conserved Process

Replication

Information

DNA

Transcription (RNA synthesis)

Information

mRNA

Translation (protein synthesis)

Ribosome

Protein

A Natural Process!!

DNA is DNA is DNA!!!
And is the “Same” in Plants and Humans

Insulin, TPA, Growth Hormone, Other Drugs For Human Health in Bacterial Factories!!

Has Lead to Genetic Engineering & Biotechnology
So Has Genetic Engineering in Plants....

With Unpredictable Results in the Beginning...
Modern Plant Genetic Engineering is Less Than 30 Years Old!

June 30, 1981

Protein Gene Is Transplanted From Bean to Sunflower

UPI

August 29, 1986

GENE-ALTERED PLANT TO GET TEST

AP

The crop will consist of only 20 plants, but experts say the tiny tobacco stand may lead to an inexpensive genetic way to fight costly plant-devastating insects.

The Rohm & Haas Company of Philadelphia, one of the world's largest producers of chemicals, announced Wednesday that the United States Department of Agriculture had approved the world's first field test of genetically altered caterpillar-resistant plants. The Agriculture Department confirmed that the approval had been granted.

Two other chemical companies, Ciba-Geigy and Agracetus, have been conducting similar tests with genetically altered plants resistant to weeds.

September 3, 1987

COMPANY NEWS; Insect-Resistant Plant Reported

REUTERS

LEAD: A Belgian company said it had made an important scientific breakthrough by altering plants genetically so they became poisonous to insects. Plant Genetic Systems of Ghent said its technique could result in a big reduction in the spraying of farm crops with insecticides.

A Belgian company said it had made an important scientific breakthrough by altering plants genetically so they became poisonous to insects. Plant Genetic Systems of Ghent said its technique could result in a big reduction in the spraying of farm crops with insecticides.

P.G.S. said field trials of tobacco plants altered with the gene of a natural, non-toxic insecticide showed that successive generations of the plants produced enough of the insecticide in their leaves to kill caterpillars.
Before There Was Dolly the Sheep There Were Cloned Orange Carrots!!!
Plant Genome Projects Are Identifying Genes Essential For Increasing Crop Yields!!

**Plant Genomes Sequenced To Date**

- Arabidopsis
- Rice
- Poplar Tree
- Soybean
- Corn
- Medicago
- Papaya
- Grape
- Castor Bean
Gene Engineering Techniques Can Also Be Used To Transfer Specific Genes Into Crops

TRADITIONAL PLANT BREEDING

Plant Breeding Combines Many Genes At Once

Desired Gene

(Many Crosses)

Mutagenesis/Selection/Genetic Diversity

New Variety

Many Genes Transferred

PLANT BIOTECHNOLOGY

Biotechnology Adds A Single Gene

Gene Transfer (one generation)

Genome Projects/Precise Sequences

Desired Gene

Commercial Variety

New Variety

One Gene Transferred

Conclusion: Plant Genome Projects & Genomics Allow Us to Identify Genes That Can Be Used to Improve Crops Plants Using Classical & Genetic Engineering Approaches
Genetic Engineering Has the Advantage of Allowing Everything That's Possible Biologically To Be Achieved

We Are Only Limited By Our Imagination and Knowledge of Biological Processes
Specific Examples of Bioengineered Crops
Pest Resistance
How to Control Insects?

Southern California Checklist

PROTECT CABBAGE CROPS. The minute you plant a brassica, squadrons of cabbage white butterflies seem to descend on it to lay their eggs. The easiest way to thwart them is to cover your cabbage crops with row covers right from the start. The next best option is spraying with Bacillus thuringiensis to kill the young caterpillar larvae.

Bt Has Been Used For Many Years To Control Pests by Conventional And Organic Farmers !!!
How to Make an Insect-Resistant Plant

1. Isolate bacterial gene that produces protein toxic against certain insects

2. Insert Bt gene and a "marker" gene into cells

3. Identify cells with Bt and "marker" genes

4. Allow cells to grow into plants. Plants now produce toxins against insect pests

BACILLUS THURINGIENSIS (BT) TOXIN GENE FROM BACTERIUM MARKER GENE DYING PLANT CELL THAT DID NOT TAKE UP GENES PLANT CELL THAT DID TAKE UP GENES DESTRUCTIVE PEST BT TOXIN DEAD PESTS
INSECT RESISTANCE with Bt

CONTROL  BT
Specific Examples of Bioengineered Crops

Abiotic Stress
Identifying Genes For Drought and Freezing Tolerance
Major Factors in Lowering Crop Yield

Using a **Wild Potato Gene** to Engineer Potato Plants Resistant to Potato Blight Fungus

**Gene RB Cloned From Solanum bulbocastanum Confers Broad Spectrum Resistance to Potato Late Blight** Song et al., PNAS 100, 9128-9133 (2003)

Potato Blight Caused the Irish Famine That Killed One Million People in the Late 19th Century and Resulted in a Large Migration of Irish People to the United States!!!
Identifying Salt Tolerant Genes

Sanan-Mishra et al. PNAS 102, 509-514 (2005)
Specific Examples of Bioengineered Crops

Seeds
So......Why Seeds??

Seeds Protect and Disperse Plant Embryos and Come in Many Shapes and Sizes!
Seeds Are Used in Many Ways as Food, Beverages, Spices, and Fuels!

Beans
Peas
Wheat
Corn
Coconut
Cashew Nuts
Peanuts
Pecans
Cocoa Beans
Coffee Beans
Nutmeg
Mustard
Most Importantl…y Our Food is Derived From Fourteen Crops & Over Half Produce Seeds For Human and Animal Consumption

Seed Crops
- Wheat
- Rice
- Corn
- Barley
- Sorghum
- Soybean
- Common Bean
- Coconut

Non-Seed Crops
- Potato
- Sweet Potato
- Cassava
- Sugar Beet
- Sugar Cane
- Banana

In Some World Populations 75% of Calories Are Derived From Seeds!
Vitamin A Deficiency Causes 1,000,000 Deaths Per Year!

Nutritionally-Enhanced Rice Seeds
Engineering For Seed Size & Yield Is Not New!

Our American Ancestors, 10,000 BC

Engineering Bigger Seeds
10,000 Years Ago

- Elder
- Sunflower
- Squash

Wild  Crop

Engineering Bigger Seeds
Today

WT  ap2-10

But Need to Identify the Critical Genes

Jofuku et al., PNAS, 2005
Specific Examples of Bioengineered Crops

Biofuels
Using Dedicated Energy Crops To Produce Biofuel

“With plausible technology developments, biofuels could supply some 30% of global demand in an environmentally responsible manner without affecting food production. To realize that goal, so-called advanced biofuels must be developed from dedicated energy crops, separately and distinctly from food.”

Steven E. Koonin
Chief Scientist, British Petroleum
Biomass Yield Matters

- At 2 tons of biomass per acre, a 5,000-ton/day biorefinery would require a radius of about 50 miles to support it.
- A 20-ton dedicated energy crop would shrink that area by 90%
Potential Dedicated Energy Crops

- Miscanthus
- Switchgrass
- Sugarcane
The “Perfect” Energy Crop

- **High biomass:** increased growth rate, photosynthetic efficiency, delayed flowering
- **Improved composition & structure:** higher fuel yield per ton
- **Disease and pest resistance**
- **Optimized architecture:** dense planting, no lodging, easier harvest
- **Salt, pH and Aluminum tolerance**
- **Deep roots:** drought tolerance, nutrient uptake, carbon sequestration
- **Rapid and cost-effective propagation**
- **Stand establishment:** cold germination, cold growth
- **Perennial:** multi-year crop, efficient nutrient use, high fossil energy ratio
- **These Are All Yield Traits!**

These traits ensure efficient nutrient use and high fossil energy ratio, making the "Perfect" Energy Crop a sustainable and high-yielding option.
Engineering Biomass
10,000 Years Ago

Foxtail Millet

Wild  Domesticated

Engineering Biomass
2008

35S:ANT
Plants Have Been Engineered For Large Numbers of Traits in Laboratories Around the World
Tens of Thousands of GE Experiments!!

Genetically Engineered Traits

- Improving Pest and Weed Management
  - Herbicide tolerance
  - Virus resistance
  - Insect resistance
  - Bacterial resistance
  - Fungal resistance
  - *Chemical Free* Crops

- Improving Agronomic Properties
  - Altering cold sensitivity
  - Improving water stress tolerance
  - Improving salt tolerance
  - Improving nutrient uptake
  - Drought Resistance*

- Improving PostHarvest Qualities
  - Delay of fruit ripening
  - Delay of flower senescence/timing
  - High-solids tomatoes
  - High-starch potatoes
  - Sweeter vegetables

- Improving Plant Breeding
  - Male sterility; production of hybrid seeds

- Improving Nutritional Quality
  - High-methionine and high-lysine seeds
  - Decaffeinated Coffee*
  - Vitamin-enriched grains
  - Allergen-free seeds/grains*

- Molecular Farming
  - Oils
  - Starch
  - Plastic
  - Enzymes, Pharmaceuticals
  - Ethanol/Transportation Fuel*

- Detoxifying Contaminated Soils

Crops in Desert & Drought
Crops as Factories & *Vaccines*
*Healthier* Crops
Crops to Clean Environment

But Only a Few Have Helped Generate New Crops!
The “Simple Ones With Economic Drivers”
One Way is to Use These New Traits in Engineered Crops That Farmers Have Adopted Faster Than Any New Agricultural Technology In the Past 100 Years!

Over One Billion Acres of Bioengineered Crops Have Been Grown World-Wide Since 1996 and 250 Million Acres in 2007
Engineered Crops Have Increased Yields, Reduced Pesticide Use, and Increased Incomes of Farmers in the Developing World

However... There's a battle raging to get bioengineered crops adopted in many parts of the world.
Professor Frank Furedi, University of Kent, England
The End

....or is it the Beginning?
THE BEGINNING: Learning Molecular Biology Techniques and Choosing Arabidopsis Transcription Factor Genes to Knock Out

THE END: Preparing a Power Point Presentation of Results and Giving a Talk at the Final Class Symposium

HC70AL Gene Discovery Laboratory

What Are the Functions of Transcription Factor Genes That Are Active During Embryo and Seed Development? (WebBOOK)

Using DNA Sequencing Analysis to Verify the T-DNA Insert Sites in the T-DNA Knock-Out Genes

Using PCR to Screen Plant Populations for Knock-Outs in Transcription Factor Genes

Using RT-PCR and GeneChip Analysis to Study Transcription Factor Gene Activity in Seeds and Other Plant Organs

Cloning Promoters of Transcription Factor Genes Chosen to Be Knocked Out and Using Green Fluorescent Protein to Study Their Activity During Seed Development

Using a Dissecting Microscope and a Microscope with Nomarski Optics to Examine the Phenotype of Embryos and Seeds in Knock-Out Lines
Going Long Distance
Winter 2009!

A Model For Cross-Campus Interactive Learning