Biotechnology Applications for Plant Breeding and Genetics
Prerequisite: CSS 350 or ZOL 341.

Principles, concepts, and techniques of agricultural plant biotechnology.

Recombinant DNA technology, plant molecular biology, transformation, cell tissue, and organ culture in relation to plant improvement.

Biotechnology Applications for Plant Breeding and Genetics

“To learn by doing”
LAB ORIENTATION

Instructors:

Dr. David Douches
Professor & Director of Plant Breeding & Genetics Program
Phone: 355-0271 ext. 1198
E-mail: douchesd@msu.edu

Dr. Guo-qing Song
Visiting Assistant Professor, Department of Horticulture
A343, PSS Bldg
Phone: 355-5191 (Ex. 1399)
E-mail: songg@msu.edu

Teaching Assistant:

Sanghyuck Park, E-mail: parksa31@msu.edu

Text Book:

Gene Cloning and DNA Analysis An Introduction—5th Edition,
by T.A. Brown (The Student Book Store)
COURSE OBJECTIVES:

• To inform the student on the cellular and molecular techniques of plants, with emphasis on genetic engineering of plants as it relates to crop improvement activities.

• To inform the student of safe laboratory practices in the conduct of plant cell and molecular experiments.

EXPECTATIONS OF STUDENTS:

• Attend class and be on time.
• Complete assignments.
• Be prepared by reading required readings.
• Leave room if need to make a cell phone call.
• No texting during lectures.

GRADING:

The course grade will be computing on the basis of student performance and participation in four areas:

Home work (problem sets 15%, lab exercises 5%, database II 5%)  25%
Laboratory notebook evaluation 10%
Components (purpose, M&M) 15%
Contents (requisite experiments) 75%
Style 5%
Neat & Organized 5%
Semester exams (midterm I, II, 25% each) 50%
Term paper 15%
Extra credit up to 5% for best term paper presentations.
All students are expected to keep a laboratory notebook with a detailed record of the results, a discussion of the results, and general conclusion draw from the experiments including suggestions for improving the experiment where appropriate.
<table>
<thead>
<tr>
<th>Date</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 13</td>
<td>Course and laboratory orientation; Sterile techniques and tissue culture</td>
</tr>
<tr>
<td>January 20</td>
<td>Tobacco transformation and Plasmid DNA isolation</td>
</tr>
<tr>
<td>January 27</td>
<td>Subculture; Plant DNA isolation (small prep kits)</td>
</tr>
<tr>
<td>February 3</td>
<td>DNA quantification and PCR for transgene detection</td>
</tr>
<tr>
<td>February 10</td>
<td>Agarose gels; Reporter genes; Guest speaker (Joe Coombs) and Insect bioassay</td>
</tr>
<tr>
<td>February 17</td>
<td>Genomic DNA isolation and PCR for fingerprinting</td>
</tr>
<tr>
<td>February 24</td>
<td>Database; DNA digestion using enzymes; electrophoresis for PCR; Midterm exam I</td>
</tr>
</tbody>
</table>

**Spring break March 2-6**

<table>
<thead>
<tr>
<th>Date</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 10</td>
<td>DNA digestion gel: Southern blot concept and transfer; term paper concept</td>
</tr>
<tr>
<td>March 17</td>
<td>Probe labeling; DNA hybridization</td>
</tr>
<tr>
<td>March 24</td>
<td>Non radioactive detection; Vector construction (DNA extraction from gels and ligation)</td>
</tr>
<tr>
<td>March 31</td>
<td>E. Coli (DH5α) transformation; Genomics Tech Facility Visit; Review Southern Result</td>
</tr>
<tr>
<td>April 7</td>
<td>Review DH5α transformation results; Genetic markers (SNAPs); Class Presentation; and High throughput marker lab visit (Sugarbeet and Dry bean labs)</td>
</tr>
<tr>
<td>April 14</td>
<td>Real Time-PCR lecture and demo; and Class presentations</td>
</tr>
<tr>
<td>April 21</td>
<td>Genetic maps and RNA isolation; Turn in the term paper</td>
</tr>
<tr>
<td>April 28</td>
<td>RT-PCR; RNAi; and Protein detection Midterm exam II</td>
</tr>
</tbody>
</table>
Lab Setup

1. Please form 9 groups
2. Each group has 3 students
3. Write down the groups and remember your group number
4. Each group should get their own supplies from the cabinet but share Pipetman
5. Please follow ALL lab instruction for your safety
Lab Safety

1. No eating, drinking or smoking in the laboratory AT ANY TIME.

2. Apparel: Shoes with close toed are to be worn at all times. We will be using substances that can ruin your clothes, e.g. bleach, so we have lab coats, you may want to wear it. We will not be using many dangerous chemicals, but safety glasses will be provided when we are using acids, etc.

3. Spills: If you spill a chemical on yourself, wash immediately with copious amounts of water and notify the TA or me. In the event of a spill on the floor or a bench involving hazardous materials (such as strong acid or base or a volatile organic compound) notify us immediately and receive instructions regarding cleanup before attempting to clean it up yourself.

4. Accidents: Be careful! Pay attention to what you are doing at all times. If you injure yourself in the laboratory in any way (however minor you may think the injury is), report it to us immediately.

5. Broken glass: Do not ever put any glass in the regular trash can. Everybody breaks glass occasionally. If you break something, don't rush to clean it up with your hands. Find a broom or dust brush, sweep up the glass and place it in the appropriate broken glassware container.

6. Waste: Do not put any waste chemicals down the sink. We will instruct you as to disposal. All transgenic material must be autoclaved before disposal. We will also be using sharps, e.g. needles, and other "hospital" supplies, e.g. syringes These must be disposed of in a special container, not in the trash.

7. Pipetting: Mouth pipetting is forbidden. Use pipettors at all times.

8. Gloves: Wear gloves when you handle some dangous chemicals or bacterium. Do not touch items around with your contaminated gloves.

9. Volatile chemicals: Use the fume hood when working with volatile chemicals. Check to make sure the hood is working before opening the volatile chemical.

10. Dirty labware: Follow the TAs instructions on how to deal with dirty labware.

11. Labeling: Make sure that all of your cultures, etc. are properly labeled and materials are stored where instructed.

12. Transgenic procedures: Genetic manipulation experiments must be carried out in accordance with guidelines laid down by National Institutes of Health, and our local Environmental Health and Safety on our campus. We will instruct you in this.

13. If cell phone is on in the classroom & lab, then it must be on vibrate or silent. Take all calls out of classroom.
Definition: *Bio* = life and *technology* = applying science to solve a problem

**Bio-tech-nol-o-gy, noun (1941):** A collective term for a variety of scientific techniques that use living cells or components of cells to improve crops, animals, or microorganisms.

A definition of biotechnology from the U.S. Office of Technology Assessments reads,

"Any technique that uses living organisms to make or modify products, to improve plants or animals, or to develop microorganisms for specific purposes."

Most people connect the word biotechnology with the idea of moving genes from one plant or animal or microbe to another, because genetic engineering is an important tool for a biotechnologist.
### Branches:

<table>
<thead>
<tr>
<th>Biotechnology branches</th>
<th>Genetic engineering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diagnostic techniques</td>
</tr>
<tr>
<td></td>
<td>Cell/tissue techniques</td>
</tr>
</tbody>
</table>

### Applications:

<table>
<thead>
<tr>
<th>Biotechnology applications</th>
<th>Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medicine</td>
</tr>
<tr>
<td></td>
<td>Food processing</td>
</tr>
<tr>
<td></td>
<td>Bioremediation</td>
</tr>
<tr>
<td></td>
<td>Energy production</td>
</tr>
</tbody>
</table>
PLANT TRANSFORMATION - Why Alter Plants?

1. **Plant Productivity**
   - Tolerance to stress, pathogens, herbicides.

2. **Nutritional Value**
   - Proteins
   - Amino Acids
   - Lipids

3. **New & Modified Proteins**
   - Recombinant Protein Production

4. **Agronomic Traits**
   - Seed Dormancy
   - Disease Resistance
     - Bacterial
     - Fungal
   - Nematode Resistance

5. **Metabolic Pathway Regulation**
   - Nutrient Capture
   - Carbohydrate Production
   - Essential oil Production

6. **Novel Traits**
   - Fruit Ripening
   - Flower Colour

7. **Phytoremediation**
   - Pollution Removal
   - Salt Tolerance

8. **Pharmaceutical Compounds**
   - Antibodies
   - Vaccines
   - Therapeutic proteins

Adapted from Newell, 2000
Traditional Plant Breeding

Commercial cultivar  Donor plant  New germplasm or cultivar

Crosses  Desired gene  Desired gene

Plant Breeding via genetic transformation

Commercial cultivar  Donor gene  New cultivar

Gene transformation  Desired gene  Desired gene
### Traditional vs. Transgenic Breeding

<table>
<thead>
<tr>
<th>Traditional Breeding</th>
<th>Transgenic Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novel proteins may be introduced from closely-related plant species. Highly heterozygous nature; Lengthy intra- and inter-specific crosses; Limitations of the available germplasms.</td>
<td>Novel proteins may be introduced from ANY species. Vegetative propagation nature is a unique advantage for woody plants</td>
</tr>
<tr>
<td>LITTLE control over how or where a gene is expressed.</td>
<td>PRECISE control over how or where a gene is expressed.</td>
</tr>
<tr>
<td>Many genes exchange.</td>
<td>Only one gene added or inactivated.</td>
</tr>
<tr>
<td>Some unsafe traits can be bred out.</td>
<td>Increased number of ways to make foods safer.</td>
</tr>
</tbody>
</table>
GM Plants - Milestones

First genetic transformation of plant cell: petunia.

First approval for field test of insect-protected cotton.

First FDA approval for a whole food produced through biotechnology: FLAVRSAVR™ tomato

The FDA declares that biotech foods are "not inherently dangerous"

First weed- and insect-resistant biotech crops commercialized

23 biotech crop countries. Biotech crop area grew 12 percent or 12.3 million hectares to reach 114.3 million hectares

First approval for field-test of modified food plants: virus-resistant tomatoes.

Transgenic plants resistant to insects, viruses and bacteria are field-tested for the first time.
Golden Rice accumulates provitamin A (β-carotene) in the grain (http://www.goldenrice.org/)
Biofuels are transportation fuels produced from biomass.

First-generation biofuels are produced in two ways. One way is through the fermentation of either starch-based food products — such as corn kernels — or sugar-based food products — such as sugar cane — into ethanol. Another way is by processing vegetable oils, such as soy, rapeseed and palm, into biodiesel.

Second-generation biofuels are made from a wider variety of nonfood sources, such as cellulose, algae and recovered waste products. These fuels have the potential to be created from renewable resources such as switchgrass, forest and agricultural residues, municipal solid waste, and new energy crops.

In the United States, the Energy Independence and Security Act of 2007 set a mandatory Renewable Fuel Standard requiring fuel producers to use at least 36 billion gallons of biofuels by 2022. This increase in renewable fuels is projected to represent roughly 5 percent of the total U.S. gasoline consumption. Most of this increase is expected to be ethanol.
Global Area of Biotech Crops, 1996 to 2007: Industrial and Developing Countries (Million Hectares)

Source: Clive James, 2008
Global Area of Biotech Crops, 1996 to 2007: By Crop (Million Hectares)

Source: Clive James, 2008
Global Area of Biotech Crops, 1996 to 2007: By Trait (Million Hectares)

Source: Clive James, 2008
Global Adoption Rates (%) for Principal Biotech Crops (Million Hectares) 2007

Source: Clive James, 2008
Biotech Crop Countries and Mega-Countries, 2007

13 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.

Source: Clive James, 2007
GLOBAL AREA OF BIOTECH CROPS
Million Hectares (1996 to 2007)

Increase of 12%, 12.3 million hectares (30 million acres), between 2006 and 2007.

Source: Clive James, 2007.
Chapter 15

Gene Clone and DNA Analysis in Agriculture

Chapter 15.1 & 15.2 p323-340
Debate on Genetic Engineering

**Scaring?**
“Some genetic engineers have turned out to be!”
By Sam Gross, 1991

**Hoping?**
“Years ago, there was only one Santa Claus. Now because of genetic engineering, there can be lots of them.”
By Sam Gross, 1997

**Confusing?**
Genetic engineering got us into this mess, and genetic engineering will get us out of it.”
By Sam Gross, 1999
Are transgenic plants intrinsically less safe than plant varieties produced by traditional genetic crosses?

It is important to recognize that many segments of the biotech industry involve non-food crops. However, in food crops, there are many strategies to ensure safety in transgenic plants.
Steps in Plant Genetic Engineering

1. Gene Identification
   - DNA Microarray

2. Gene Cloning
   - Promoter
   - Protein coding region
   - cDNA
   - Cloning vector
   - Binary vector
   - SMG1
   - SMG2

3. Gene Transformation
   - Delivery systems
     - Via Agrobacterium or Biolistics

4. Selection and regeneration
   - Molecular verification of gene presence & expression
     - PCR
     - Southern blot
     - Gus assay

5. Greenhouse & Field Test
<table>
<thead>
<tr>
<th>Date</th>
<th>Gene Identification</th>
<th>Gene Cloning</th>
<th>Gene Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 13</td>
<td></td>
<td></td>
<td>Sterile techniques and tobacco micropropagation</td>
</tr>
<tr>
<td>Jan 20</td>
<td></td>
<td>Plasmid DNA isolation</td>
<td>Tobacco transformation</td>
</tr>
<tr>
<td>Jan 27</td>
<td>Plant DNA Isolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb 3</td>
<td>DNA quantification and PCR for transgene detection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb 10</td>
<td>Agarose gels</td>
<td>Reporter genes</td>
<td></td>
</tr>
<tr>
<td>Feb 17</td>
<td>Genomic DNA isolation and PCR for fingerprinting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb 24</td>
<td>Database; DNA digestion; Electrophoresis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Spring break

<table>
<thead>
<tr>
<th>Date</th>
<th>Gene Identification</th>
<th>Gene Cloning</th>
<th>Gene Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar 10</td>
<td></td>
<td></td>
<td>Southern blot</td>
</tr>
<tr>
<td>Mar 17</td>
<td></td>
<td></td>
<td>Tobacco transformation, Southern blot</td>
</tr>
<tr>
<td>Mar 24</td>
<td>DNA extraction from gels and ligation</td>
<td></td>
<td>Southern blot</td>
</tr>
<tr>
<td>Mar 31</td>
<td>Genomics</td>
<td>DH5 α transformation</td>
<td></td>
</tr>
<tr>
<td>Apr 7</td>
<td>Genetic markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr 14</td>
<td>Real Time-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr 21</td>
<td>Genetic maps and RNA isolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr 28</td>
<td>Genetic maps; RNAi; and Protein detection</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Learning Objectives

1. A general understanding of the various categories of plant biotechnology
2. Understand the importance of genetic engineering in crop improvement
3. Know several applications of genetic engineering
4. Be familiar with the methods for plant transformation
5. Be able to explain tissue culture, cloning, and related techniques to others
6. Grow, maintain, and propagate specific plant materials in a sterile environment
7. Describe plant genome organization and the mechanisms of gene expression in plants
8. Be able to solve simple genetics problems
9. Know how crop yields and quality can be enhanced using genetic manipulation
10. Learn basic DNA & RNA manipulations for plant science applications