

PBG 607: (Modern Techniques in Plant Breeding) 3(2-1)

Session # 1: Tutorial

Introduction to genetic engineering and plant biotechnology

Genetic engineering started way back in 1970s, when an international race started to understand the mechanism by which *Agrobacterium tumefaciens* caused plant cells to grow rapidly into a gall that produced its favorite substrates (opines).

Agrobacterium:

Agrobacterium is Gram-negative bacteria established by H. J. Conn. Economically, *A. tumefaciens* is a serious pathogen of various economically important crops including sugar beets, horse radish, walnuts, grape vines, nut trees, stone fruits, sugar beets, horse radish. This bacteria form tumors or galls which harmful for perennial crops as shown in figure below;



Source: Google images

During genetic transformation the opine-synthesis genes and tumor-promoting genes (harmful genes) are removed from the T-DNA and replaced with a gene of our own interest. In addition to gene of interest a gene for selection or antibiotic resistance gene is also incorporated into to the Plasmid.

Most commonly selection markers include;

- neomycin phosphotransferase
- hygromycin B
- Phosphinothricin acetyltransferase (which acetylates and deactivates phosphinothricin, a potent inhibitor of glutamine synthetase)
- Basta or Bialophos

Agrobacterium-mediated T-DNA transfer is widely used as a tool in plant [biotechnology](#).

Since 1996, *Agrobacterium tumefaciens* has been used for introducing desirable genes into plants to develop transgenic plants.

Agrobacterium is then used as a vector to transfer the engineered T-DNA into the plant cells where it integrates into the plant genome. This method can be used to generate transgenic plants carrying a foreign gene. *Agrobacterium tumefaciens* is capable of transferring foreign DNA to both monocotyledons and dicotyledonous plants efficiently while taking care of critically important factors like the genotype of plants, types and ages of tissues inoculated,

kind of vectors, strains of *Agrobacterium*, selection marker genes and selective agents, and various conditions of tissue culture.

Current Status of GM crops in world:

First commercial crop was tomato which was planted in 1994. The year 1996 was the first year in which a significant area [1.66 million hectares (ha)] of GM crops with different traits were planted. The area GM crops is increasing dramatically as shown in graph below:

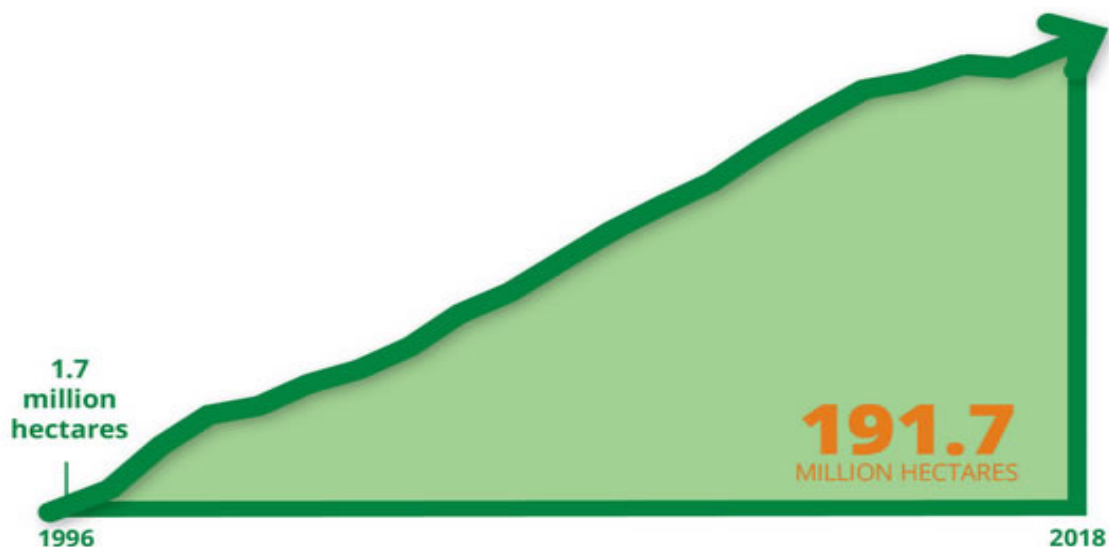
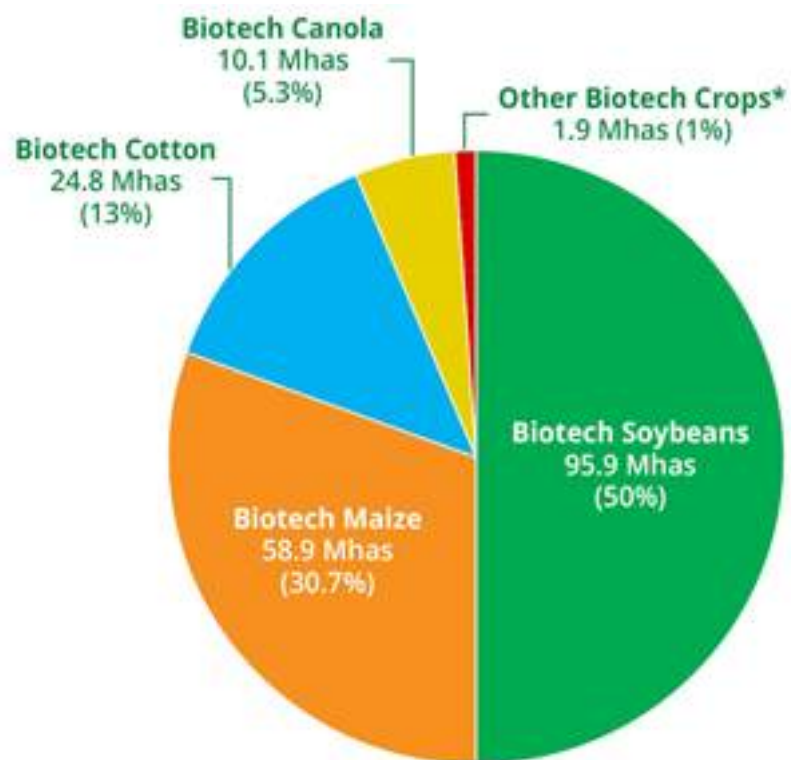


FIGURE 1. GLOBAL AREA OF BIOTECH CROPS, 1996 TO 2018 (MILLION HECTARES).

Source: ISAAA, 2018

Crop wise Status of GM crops in world:



* Biotech sugar beets, potatoes, apples, squash, papaya, and brinjal/eggplant.

BIOTECH CROPS IN 2018 (AREA AND ADOPTION RATE)

Source: ISAAA, 2018

Session # 2 & 3: Laboratory

Safety measures in the biotech laboratory:

A standard list of basic laboratory safety rules which should be followed in every laboratory that uses hazardous materials or processes. These basic rules provide behavior, hygiene, and safety information to avoid accidents in the laboratory. Laboratory specific safety rules may be required for specific processes, equipment, and materials, which should be addressed by laboratory specific SOPs.

Basic safety rules for laboratory conduct should be observed whenever working in a laboratory. Many of the most common safety rules are listed below.

- Know locations of laboratory safety showers,
 - eye washstations,
 - and fire extinguishers. The safety equipment may be located in the hallway near the laboratory entrance.
 - Know emergency exit routes.
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- No food or drink is allowed in lab unless food or drinks are provided as a part of the lab. Even though lab tables and counters are wiped down before each lab set up, as a result of some laboratory exercises, chemical residues may be present on the

tables. For labs exercises involving food or drinks, lab assistants and instructors will follow procedures that allow safe consumption. Smoking is prohibited in all University buildings.

- Shoes must be worn in lab.
- If you have very long hair, please tie it back in the laboratory.
- Handle chemicals, reagents, and stains carefully and follow all warnings. All bottles and containers are labeled as to contents and potential hazards. If, for example, a label says avoid contact with substance and fumes, do so. For potentially hazardous chemicals, information on the hazards, proper handling, and clean-up is provided on Material Safety Data Sheets (MSDS). These are available in the lab. It is highly recommended that you spend the first few minutes of the lab consulting the MSDS.
- Read all precautions in the laboratory manual and on labels and follow directions exactly.
- Always double-check the name of the reagent to be used and the name of the reagent you are using. Reagent bottles should remain stoppered, except when you are actually pouring solutions out of them. Always replace the stopper or lid of stock solutions or stains. Be sure to put them on the container they came from.
- Take only as much as you need and never return leftover solutions to a reagent bottle. Discard leftovers in the proper container.

- Do not use your thumb as a stopper. Swirl gently or put a piece of parafilm over the opening to mix solutions.
- Label all test tubes and other containers with contents.
- Do not pour reagents and chemicals down the sink. Dispose of these only in designated containers.
- Put plant remains in the designated containers. Do not discard solids or plant materials down the sinks. They will clog up the sink. Use specified containers for such wastes
- Be sure to use caution when using razor blades to prepare lab materials. Put the used blades into the designated container from which you obtained them. Do not leave them loose on the desks or counters. Never put razor blades in the wastepaper basket.
- If glassware accidentally becomes broken, carefully clean it up with a broom and dustpan. Dispose of the broken glass in containers labeled FOR BROKEN GLASS ONLY.
- When using hot plates, unplug them before you leave the lab.
- If you notice that the cord on a hot plate or microscope has become frayed and wires are showing, report this immediately to the instructor.
- If you are in doubt about directions for an experiment or activity or about use or disposal of materials, ask first before acting.

- Your instructor will review with you the location and, where applicable, use of the safety equipment in the laboratory including:
 - MSDS files
 - emergency phone
 - first aid kit
 - fire extinguisher
 - eyewash

OTHER LABORATORY PROCEDURES

1. When getting solutions for your experiments or stains for experiments or observations, put the containers or bottles back in their designated place after you have used them.

2. Unless directed to do so, do not eat our specimens.

Greenhouse materials, for example, are often sprayed with hazardous materials.

3. Do not waste paper towels or any other lab materials.

4. You are expected to clean up after yourselves:

- Take labels from test tubes and/or beakers.
- Wash all glassware and put it back where you found it.
- Place all dirty slides and cover slips in the designated containers.

- Leave tables clean when you leave the lab. Throw away any trash you generate. Wipe up water, other liquids, soil, and plant material. Be sure to discard extra plant material in the designated containers.
- Return the prepared slides in the proper slide tray.
- Long hair and loose clothing must be pulled back and secured from entanglement or potential capture.
- No contact lenses should be worn around hazardous chemicals – even when wearing safety glasses.
- Laboratory safety glasses or goggles should be worn in any area where chemicals are used or stored. They should also be worn any time there is a chance of splashes or particulates to enter the eye. Closed toe shoes will be worn at all times in the laboratory. Perforated shoes or sandals are not appropriate.
- Determine the potential hazards and appropriate safety precautions before beginning any work.
- If an unknown chemical is produced in the laboratory, the material should be considered hazardous.
- Do not pour chemicals down drains. Do NOT utilize the sewer for chemical waste disposal.

- Keep all sink traps (including cup sink traps and floor drains) filled with water by running water down the drain at least monthly.
- Do not utilize fume hoods for evaporations and disposal of volatile solvents.
- Perform work with hazardous chemicals in a properly working fume hood to reduce potential exposures.
- Avoid working alone in a building. Do not work alone in a laboratory if the procedures being conducted are hazardous.
- Laboratory employees should have access to a chemical inventory list, applicable SDSs, Department Laboratory Safety Manual, and relevant SOPs.
- No cell phone or ear phone usage in the active portion of the laboratories, or during experimental operations.
- Clothing made of synthetic fibers should not be worn while working with flammable liquids or when a fire hazard is present as these materials tend to melt and stick to exposed skin.
- Laboratory coats should not be stored in offices or break rooms as this spreads contaminants to other areas.
- Computers and instrumentation should be labeled to indicate whether gloves should be worn or not. Inconsistent glove use around keyboards/keypads is a source of potential contamination.

- Avoid wearing jewelry in the lab as this can pose multiple safety hazards.
- Laboratory Specific Safety Rules
- Safety rules for laboratory specific operations will be provided in appropriate laboratory SOPs.

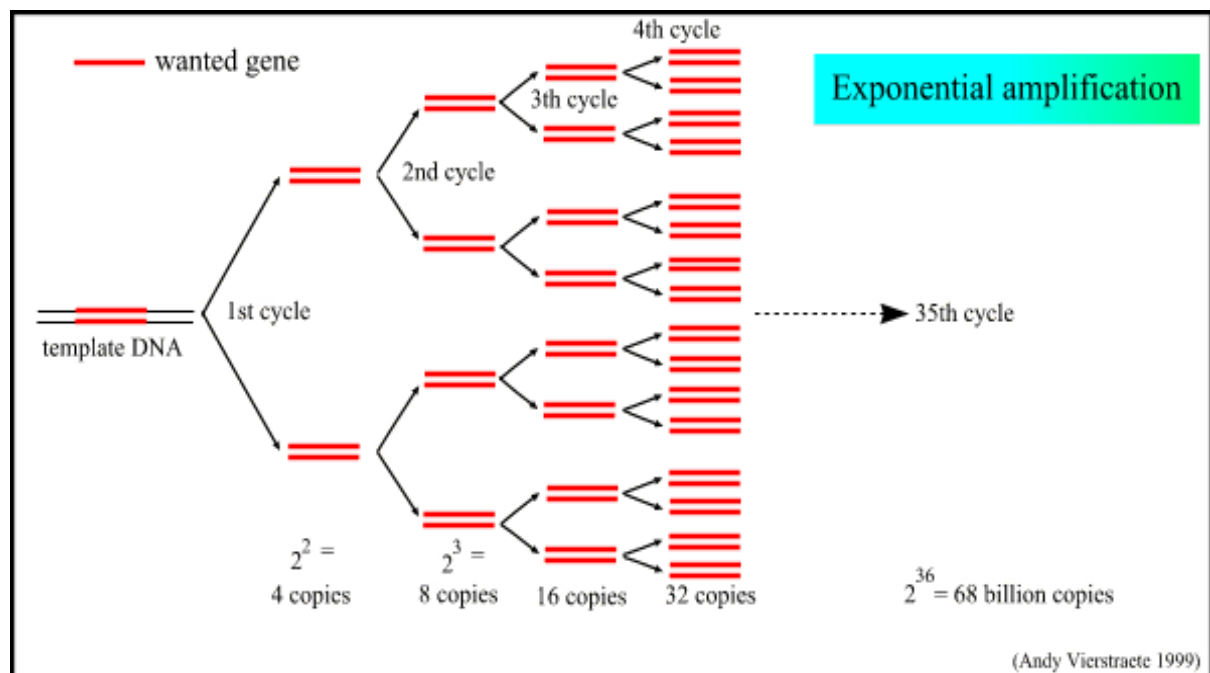
Session # 4 & 5: Tutorial

Basics of molecular biology DNA amplification and Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a method widely used in molecular biology to rapidly make millions to billions of copies of a specific DNA sample allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

PCR was invented in 1983 by Kary Mullis and he was awarded noble prize for this marvellous invention.

Using PCR, copies of very small amounts of DNA sequences are exponentially amplified in a series or cycles of temperature changes as shown below;



Components of PCR:

The reaction is commonly carried out in a small volume of 10–100 μL in mini reaction tubes (0.2–0.5 mL volumes) in a [thermal cycler](#).

A basic PCR consist of following several components and reagents:

- a *DNA template* that contains the DNA target region to be amplified
- an enzyme (*DNA polymerase*) that [polymerizes](#) new DNA strands
- Two DNA *primers*: *forward and reverse primers* that are [complementary](#) to the 3' ends of each of the [sense and anti-sense](#) strands of the DNA target specific primers that are complementary to the DNA target region.

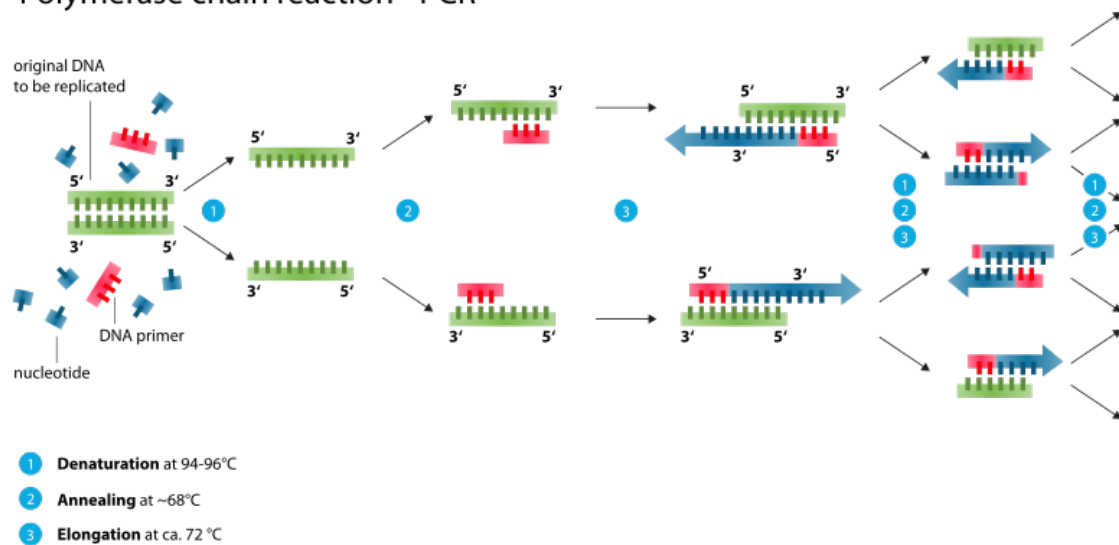
(Primers are mostly purchased from commercial biochemical suppliers)

- *deoxynucleoside triphosphates*, (dNTPs), the building blocks from which the DNA polymerase synthesizes a new DNA strand
- a [buffer solution](#) providing a suitable chemical environment for optimum activity and stability of the DNA polymerase
- [bivalent cations](#), typically [magnesium](#) (Mg) or [manganese](#) (Mn) ions; Mg^{2+} is the most common, but Mn^{2+} can be used for [PCR-mediated DNA mutagenesis](#), as a higher Mn^{2+} concentration increases the error rate during DNA synthesis^[10]; and *monovalent cations*, typically [potassium](#) (K) ions

Procedure

Typically, PCR consists of a series of 30–40 repeated temperature changes, called thermal cycles, with each cycle commonly consisting of two or three discrete temperature steps (see figure below).

Polymerase chain reaction - PCR



Initial denaturation or initialization:

The cycling is often preceded by a single temperature step at a very high temperature around 94-95 °C. At this high temperature the double stranded DNA is melted apart. The Taq polymerase enzyme is activated at this temperature. The cycle at the end holds on a constant temp @ 72 °C at the end for final product extension..

Denaturation:

This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.

Annealing:

In this step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds.

This temperature depends on the primer length and GC content of the primers. This temperature allow annealing of the primers to each of the single-stranded DNA templates.

Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand.

Extension/elongation:

The temperature depends on the DNA polymerase used; the optimum activity temperature for the thermostable DNA polymerase of Taq (Thermus aquaticus) polymerase is approximately 75–80 °C (167–176 °F),^{[13][14]} though a temperature of 72 °C (162 °F) is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that are complementary to the template in the 5'-to-3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxy group at the end of the nascent (elongating) DNA strand. The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify.

As a rule of thumb, at their optimal temperature, most DNA polymerases polymerize a thousand bases per minute. Under optimal conditions (i.e., if there are no limitations due to limiting substrates or reagents), at each extension/elongation step, the number of DNA target sequences is doubled. With each successive cycle, the original template strands plus all newly

generated strands become template strands for the next round of elongation, leading to exponential (geometric) amplification of the specific DNA target region.

The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions of copies. The formula used to calculate the number of DNA copies formed after a given number of cycles is 2^n , where n is the number of cycles. Thus, a reaction set for 30 cycles results in 2^{30} , or 1,073,741,824, copies of the original double-stranded DNA target region.

Final elongation:

This single step is optional, but is performed at a temperature of 70–74 °C (the temperature range required for optimal activity of most polymerases used in PCR) for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully elongated.

Final hold:

The final step cools the reaction chamber to 4–15 °C for an indefinite time, and may be employed for short-term storage of the PCR products.

Session # 6: Tutorial

DNA fingerprinting and molecular markers

Genetic/molecular markers:

In genetics, a **molecular marker** also identified as [genetic marker](#) is a fragment of [DNA](#) that is associated with a certain location within the [genome](#) of an organism. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA.

Types of genetic markers:

There are many types of genetic markers, each with particular limitations and strengths. Within genetic markers there are three different categories:

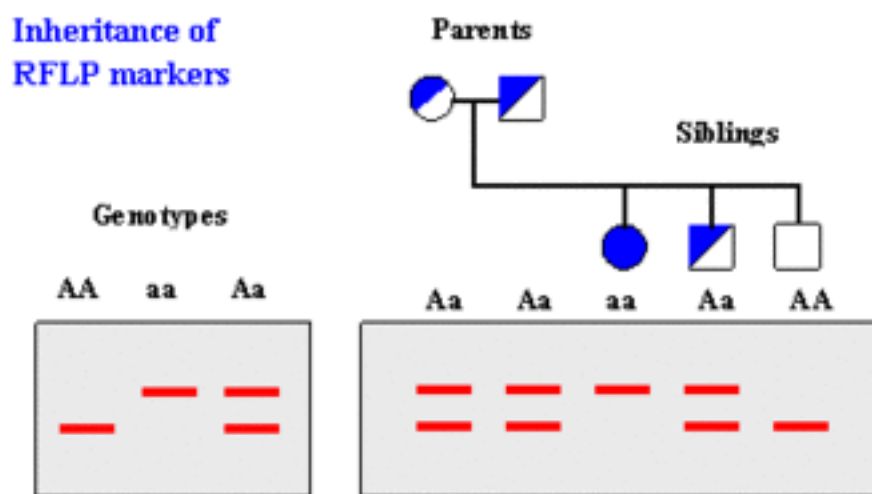
- First Generation Markers,
- Second Generation Markers", and
- New Generation Markers

Molecular markers are effective because they identify an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification. They can identify small changes within the mapping population enabling distinction between a mapping species, allowing for segregation of traits and identity. They identify particular locations on a chromosome, allowing for physical maps to be created. Lastly they can identify how many alleles an organism has for a particular trait (bi allelic or poly allelic).

List of Markers	Acronym
Restriction Fragment Length Polymorphism	RFLP
Random Amplified Polymorphic DNA	RAPD
Amplified Fragment Length Polymorphism	AFLP
Single Nucleotide Polymorphism	SNP
Allele Specific Associated Primers	ASAP
Inverse Sequence-tagged Repeats	ISTR

restriction fragment length polymorphism (RFLP):

A technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence.

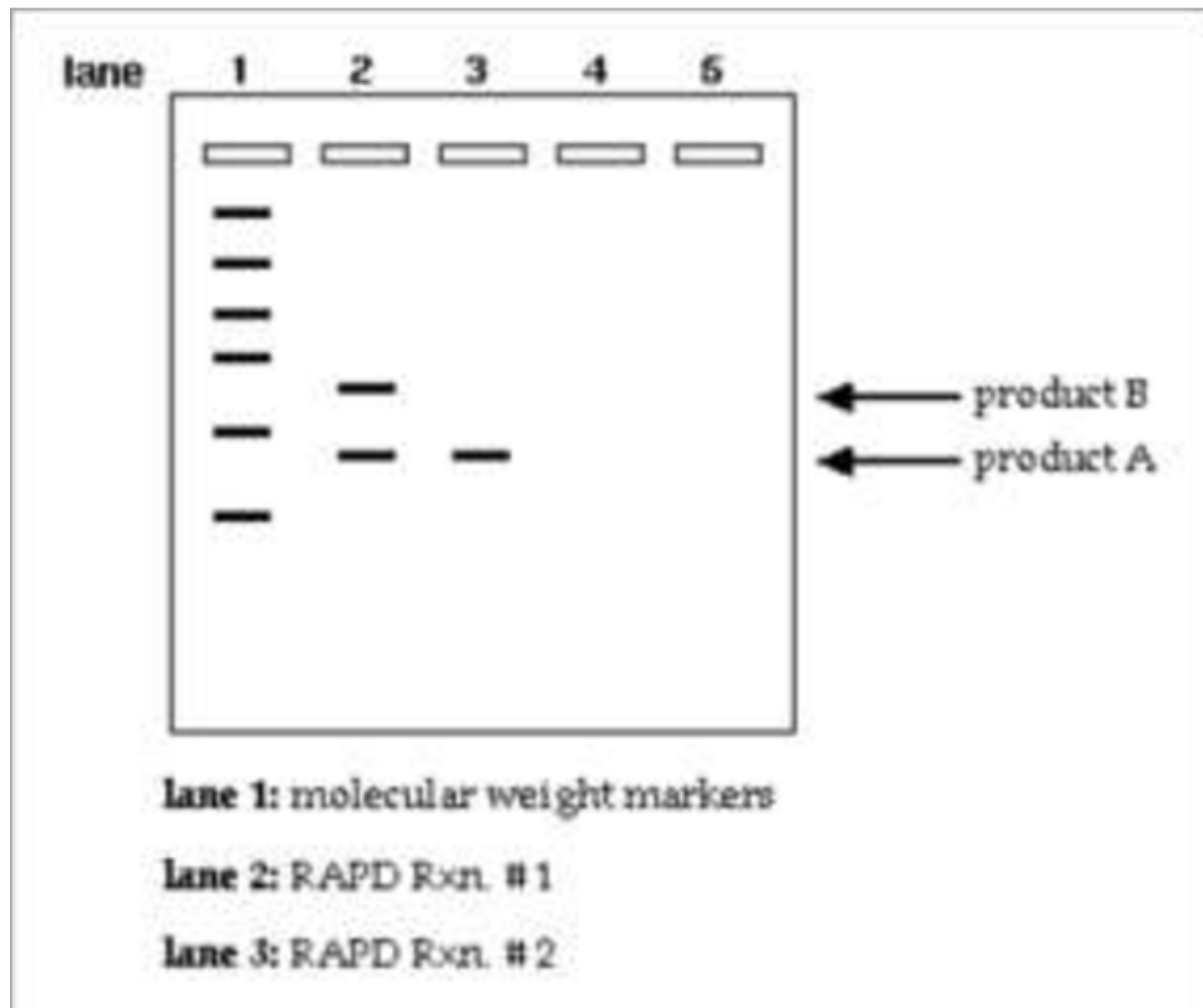


Analysis and inheritance of allelic RFLP fragments (NIH)

Random Amplified Polymorphic DNA (RAPD):

It is a type of [PCR](#) which amplifies the target fragments but the segments of DNA that are amplified are random. The scientist performing RAPD creates several arbitrary, short primers (8–12 nucleotides), then proceeds with the PCR using a large template of genomic DNA,

hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from an RAPD reaction.



amplified fragment length polymorphism:

AFLP-PCR or just AFLP is a PCR-based tool used in genetics research, DNA fingerprinting, and in the practice of genetic engineering. Developed in the early 1990s by Keygene, AFLP uses

restriction enzymes to digest genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments.

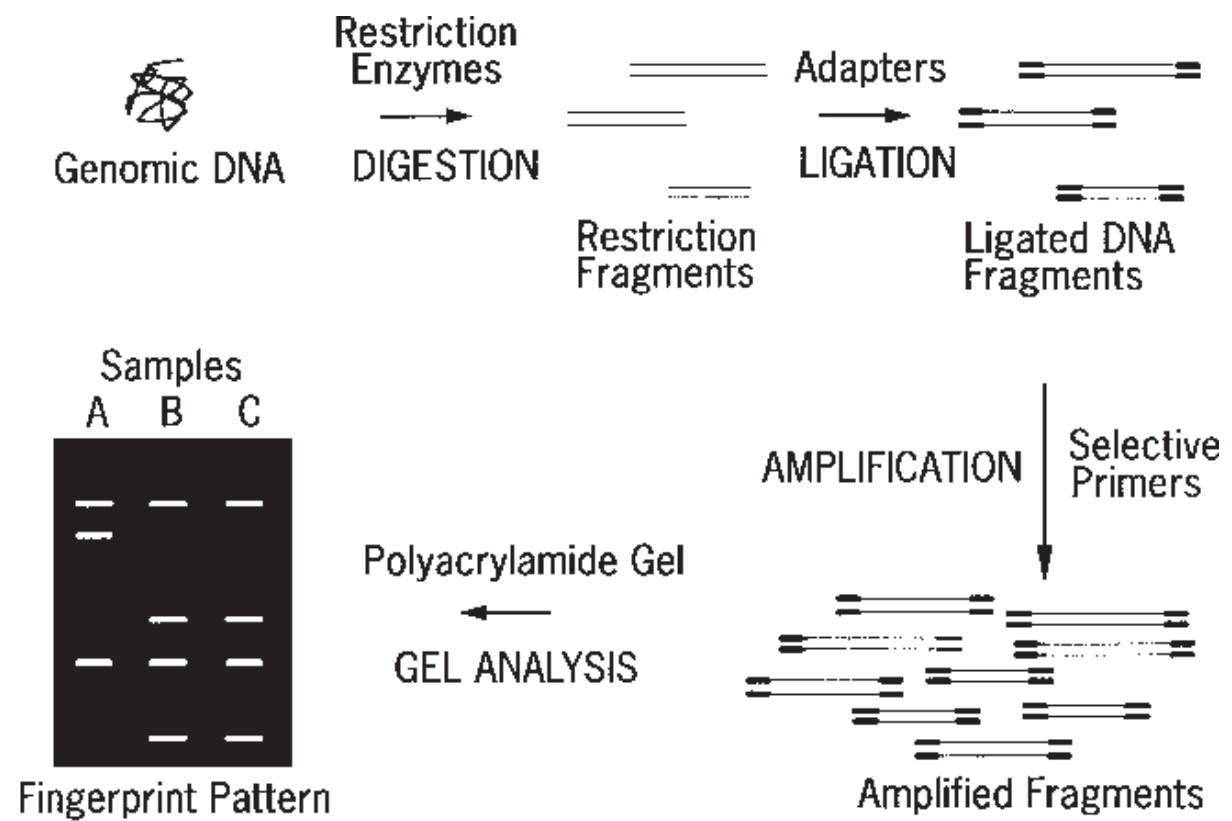


Figure 1: A schematic displaying the four basic steps of AFLP: digestion, ligation, amplification and gel analysis. Genomic DNA is digested by restriction enzymes and adaptors are ligated to the restriction fragments.

Session # 7& 8: Tutorial

Biotechnological approaches to drought tolerance, salt tolerance and protein quality in various field crops.

Genetic Engineering approaches for Drought Tolerant:

An intensive research work has been carried out on Arabidopsis which has played a vital role in the elucidation of the basic processes underlying stress tolerance, and the knowledge obtained has been transferred to a certain degree to important food plants.

Many of the genes known to be involved in stress tolerance have been isolated initially in Arabidopsis. The introduction of several stress-inducible genes into plants by genetic engineering has resulted to increased tolerance of transgenics to drought, cold and salinity stresses. Some examples are reviewed in the following section.

Manipulation of the stress response to abscisic acid (ABA)

ABA levels in the plant greatly increase in response to water stress, resulting in the closure of stomata thereby reducing the level of water loss through transpiration from leaves and activate stress response genes.

The reaction is reversible: once water becomes available again, the level of ABA drops, and stomata re-opens. Increasing the plant's sensitivity to ABA has therefore been a very important target for improving drought tolerance.

ERA1, a gene identified in *Arabidopsis*, encodes the β -subunit of a farnesyl-transferase, and is involved in ABA signaling. Plants lacking *ERA1* activity have increased tolerance to drought, however are also severely compromised in yield. In order to have a conditional, reversible down-regulation of ABA, a group of Canadian researchers used a drought-inducible promoter to drive the antisense expression of *ERA1*, in both *Arabidopsis* and canola plants¹¹. Transgenic plants performed significantly better under water stress, with consistently higher yields over conventional varieties. Importantly, there was no difference in performance between transgenic and controls in conditions of sufficient water, demonstrating that the technology has no yield-drag¹¹. Multi-location trials have confirmed yield increases due to enhanced protection to drought to be 15-25 percent compared to non-transgenic controls (<http://www.performanceplants.com>).

Performance Plants Inc, a Canadian plant biotechnology company, is developing the technology for commercialization, under the name Yield Protection Technology™ (YPT™). YPT™ is also being developed for maize, soybean, cotton, ornamentals and turf grass to be available to farmers in early 2011.

ABA-independent gene regulation to drought stress:

The transcription factors *DREB1* and *DREB2*, are important in the ABA-independent drought tolerant pathways, that induce the expression of stress response genes. Over-expression of the native form of *DREB1*, and of a constitutively active form of *DREB2*, increases the tolerance of transgenic *Arabidopsis* plants to drought, high salinity and cold. Although these genes were initially identified in *Arabidopsis* plants, their presence and role in stress tolerance have been reported in many other important crops, such as rice, tomato, barley, canola, maize, soybean, rye, wheat and maize, indicating that this is a conserved, universal stress defense mechanism in plants⁹. This functional conservation makes the *DREB* genes important targets for crop improvement for drought tolerance through genetic engineering.

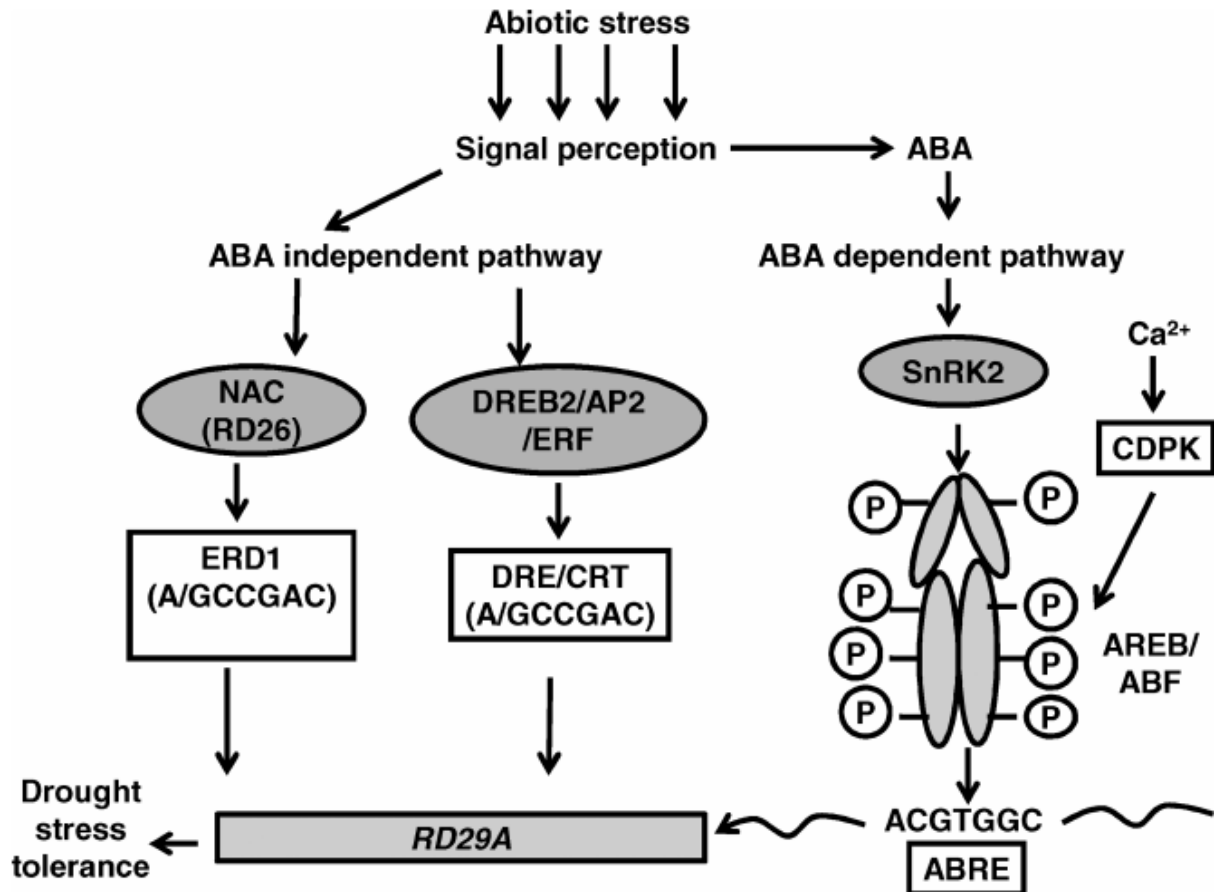


FIG. ABA-dependent and ABA-independent signaling in plants. DREB2 and NAC signal transduction pathways are induced in response to drought stress and are involved in the expression of downstream target genes responsive to drought stress both in *Arabidopsis* and maize. The NAC transcription factor and DREB2 are involved in ERD1 and RD29A gene expression, respectively. SnRK2 can interact physically and phosphorylate b-ZIP transcriptional activators that might also be phosphorylated by certain CPKs, which recognize similar or even identical C-domain motifs as the SnRK2s. The RD29 gene contains both ABRE and DRE/DRT cis elements in its promoter. DRE, drought-responsive, ABA, Absciscic acid (modified from Yamaguchi-Shinozaki and Shinozaki, 2006; Shinozaki and Yamaguchi-Shinozaki, 2007; Wasilewska et al. , 2008).

Session # 9 & 10: Tutorial

Scope of transgenic plants in plant breeding.

Since the first stably transgenic plant produced in the early 1980s and the first commercialized transgenic plant in 1995, biotechnology has revolutionized plant agriculture.

More than a billion acres of transgenic cropland has been planted worldwide. In the United States, over half of the corn and cotton and three-quarters of soybean produced are transgenic for insect resistance, herbicide resistance, or both. Biotechnology has been the most rapidly adopted technology in the history of agriculture and continues to expand in much of the developed and developing world.

Current Status of GM crops in world:

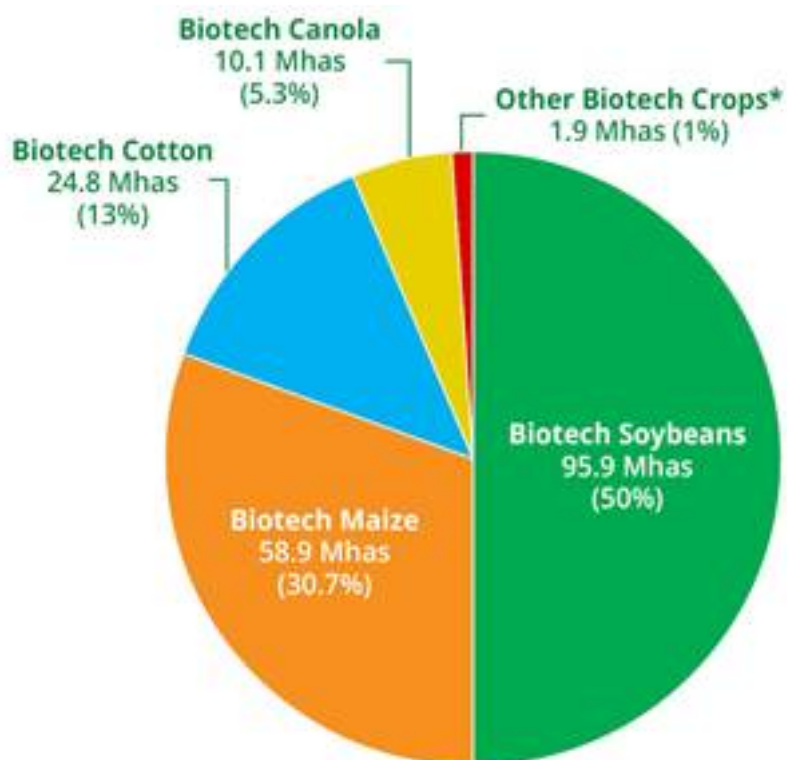
First commercial crop was tomato which was planted in 1994. The year 1996 was the first year in which a significant area [1.66 million hectares (ha)] of GM crops with different traits were planted. The area GM crops is increasing dramatically as shown in graph below:



FIGURE 1. GLOBAL AREA OF BIOTECH CROPS, 1996 TO 2018 (MILLION HECTARES).

Source: ISAAA, 2018

Crop wise Status of GM crops in world:



* Biotech sugar beets, potatoes, apples, squash, papaya, and brinjal/eggplant.

BIOTECH CROPS IN 2018 (AREA AND ADOPTION RATE)

Source: ISAAA, 2018

WHY FARMERS USE GM CROPS

The primary driver of adoption among farmers (both large commercial and small-scale subsistence) has been the positive impact on farm income. The adoption of biotechnology has had a very positive impact on farm income derived mainly from a combination of enhanced productivity and efficiency gains. In 2005, the direct global farm income benefit from GM crops was \$5 billion. If the additional income stemming from second crop soybeans in Argentina is considered,⁴ this income gain rises to \$5.6 billion. This is equivalent to having added between 3.6% and 4.0% to the value of global production of the four main crops of soybean, maize, canola, and cotton, a substantial impact. Since 1996, worldwide farm incomes have increased by \$24.2 billion or \$27 billion inclusive of second-crop soybean gains in Argentina directly because of the adoption of GM crop technology. The largest gains in farm income have arisen in the soybean sector, largely from cost savings, where the \$2.84 billion additional income generated by GM HT soybean in 2005 has been equivalent to adding 7.1% to the value of the crop in the GM-growing countries, or adding the equivalent of 6.05% to the \$47 billion value of the global soybean crop in 2005.

Session # 11 & 12: Laboratory

Introduction to aseptic techniques; autoclaving and sterilization

Autoclave Sterilization:

Autoclaves provide a physical method for disinfection and sterilization. They work with a combination of steam, pressure and time. Autoclaves operate at high temperature and pressure in order to kill microorganisms and spores.

Autoclave Sterilizers are used to decontaminate certain biological waste and sterilize media, instruments and lab ware. Regulated medical waste that might contain bacteria, viruses and other biological material are recommended to be inactivated by autoclaving before disposal.

An autoclave is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes. Autoclaves vary in size, shape and functionality. A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents.

Autoclave Sterilizers:

To be effective against spore forming bacteria and viruses, autoclaves need to have steam in direct contact with the material being sterilized (i.e. loading of items is very important).

Create vacuum in order to displace all the air initially present in the autoclave and replacing it with steam.

Implement a well designed control scheme for steam evacuation and cooling so that the load does not perish.

The efficiency of the sterilization process depends on two major factors. One of them is the thermal death time, i.e. the time microbes must be exposed to at a particular temperature before they are all dead. The second factor is the thermal death point or temperature at which all microbes in a sample are killed.

The steam and pressure ensure sufficient heat is transferred into the organism to kill them. A series of negative pressure pulses are used to vacuum all possible air pockets, while steam penetration is maximized by application of a succession of positive pulses

Autoclave Uses & Advantages:

An autoclave chamber sterilizes medical or laboratory instruments by heating them above boiling point. Most clinics have tabletop autoclaves, similar in size to microwave ovens. Hospitals use large autoclaves, also called horizontal autoclaves. They're usually located in the the Central Sterile Services Department (CSSD) and can process numerous surgical instruments in a single sterilization cycle, meeting the ongoing demand for sterile equipment in operating rooms and emergency wards.

They are important in tattoo shops, beauty and barber shops, dentist offices, veterinarians and many other fields.

Autoclave disadvantages:

Autoclave is unsuitable for heat sensitive objects.

Autoclaves Working Principle:

Autoclaves use pressurized steam as their sterilization agent. The basic concept of an autoclave is to have each item sterilized -whether it is a liquid, plastic ware, or glassware- come in direct contact with steam at a specific temperature and pressure for a specific amount of time. Time, steam, temperature, and pressure are the four main parameters required for a successful sterilization using an autoclave.

The amount of time and temperature required for sterilization depends on the type of material being autoclaved. Using higher temperatures for sterilization requires shorter times. The most common temperatures used are 121 C and 132 C. In order for steam to reach these high temperatures, steam has to be pumped into the chamber at a pressure higher than normal atmospheric pressure. Now that we have covered the basic principle of how autoclaves use pressurized steam to sterilize contaminated materials, we will now go over how autoclaves operate.



Similar to pressure cookers, steam sterilizer autoclaves work quickly and effectively because of their high temperature. The machine's temperature and unique shape make it easier to hold the heat inside much longer. The autoclave also does a great job of efficiently penetrating each piece of equipment. The autoclave's chambers are usually in the shape of a cylinder because cylindrical shapes are more equipped to handle the high pressure that is needed for the sterilization process to work. For safety reasons, there is an outside lock and a safety valve that prevents the autoclave steam sterilizer's pressure from getting too high.

Once you close the autoclave sterilizer chamber, a vacuum pump removes all the air from inside the device or it is forced out by pumping in steam. If done the first way, the sterilizer is pumped with high pressured steam to quickly raise the internal temperature. On every autoclave there is a thermometer that is waiting for the thermal sweet point, 268-273 degrees Fahrenheit, and then it starts its timer. During the sterilizing process, steam is continuously entering the autoclave to thoroughly kill all dangerous microorganisms. Once the required time of sterilization has elapsed, the chamber will be exhausted of pressure and steam allowing the door to open for cooling and drying of the contents.

Mode of Action Autoclave Sterilizers:

Moist heat destroys microorganisms by the irreversible coagulation and denaturation of enzymes and structural proteins. In support of this fact, it has been found that the presence of moisture significantly affects the coagulation temperature of proteins and the temperature at which microorganisms are destroyed.

Autoclave Working – Operation:

Place containers in the autoclave.

Check the strainer to see if it is clogged. The strainer is located on the bottom of the chamber near the door. The autoclave will not come up to pressure if the strainer is clogged.

SMALL autoclave:

rotate the handle counterclockwise. Be careful, steam burns! Step to the side and crack open the door. Allow the steam to escape from the chamber then open the door and remove your items.

LARGE autoclave:

First rotate the LARGE OUTER handle counterclockwise until it is loose. Next, rotate the SMALL INNER handle counterclockwise until the door opens. Be careful, steam burns! Step to the side and crack open the door. Allow the steam to escape from the chamber then open the door and remove your items.

As a courtesy to others needing to use the autoclave, promptly remove your items when the cycle is completed and you can easily open the door. Wear protective, heat resistant gloves when removing items.

Autoclaved waste materials are to be taken directly to the dumpster for disposal. Orange autoclave bags must be put into black trash bags before disposing in the dumpster.

Autoclave Cycles

To be effective, the autoclave must reach and maintain a temperature of 121° C for at least 30 minutes by using saturated steam under at least 15 psi of pressure. Increased cycle time may be necessary depending upon the make-up and volume of the load.

The rate of exhaust will depend upon the nature of the load. Dry material can be treated in a fast exhaust cycle, while liquids and biological waste require slow exhaust to prevent boiling over of super-heated liquids.

Liquids cycle

Liquids rely on the Liquids Cycle to avoid a phenomenon known as “boil-over.” Boil-over is simply a liquid boiling so violently that it spills over the top of its container. Boil-over will occur if the pressure in your autoclave chamber is released too quickly during the exhaust phase

of the cycle. Significant liquid volume can be lost to boil-over, and this can result in unwanted spills on the bottom of the autoclave chamber that must be cleaned up to avoid clogging the drain lines and the subsequent repair costs to the department.

To help prevent boil-over during the exhaust phase, the chamber pressure must be released slowly. This process is controlled by the sterilizer's control system. Controlling the exhaust rate allows the liquid load to cool off as the surrounding chamber pressure is decreased.

The exhaust rate for a Liquids Cycle is different from a standard Gravity or Vacuum Cycle, where the chamber pressure is released quickly. To prevent boil-over, the chamber pressure must decrease slowly to allow the temperature of the load to remain below the boiling point. If the pressure is exhausted all at once, the temperature of the load will be above its boiling point, resulting in instant and violent boiling.

(Slow Exhaust)

Material Recommended for:

Use with glass containers with vented closures; 2/3 full only

Liquid media

Nonflammable liquids

Aqueous solutions

Liquid biological waste

Solids or Dry cycle

(Fast Exhaust)

Material Recommended for:

Glassware: empty and inverted

no tight or impermeable closures

Dry hard items, either unwrapped or in porous wrap

Metal items with porous parts

Other porous materials

Gravity Cycle: Wrapped Goods or Pre vacuum cycle

(Clean: Fast Exhaust

Dirty: Slow Exhaust)

The traditional “Gravity Cycle” is the most common and simplest steam sterilization cycle.

During a Gravity Cycle, steam is pumped into a chamber containing ambient air. Because steam has a lower density than air, it rises to the top of the chamber and eventually displaces all the air. As steam fills the chamber, the air is forced out through a drain vent. By pushing the air out, the steam is able to directly contact the load and begin to sterilize it.

At the end of the cycle, the steam is discharged through the drain vent. However, the load can still be hot and possibly wet. To address this issue, gravity autoclaves can be equipped with a post-cycle vacuum feature to assist in drying the load. The sterilizer runs a normal Gravity Cycle and after the load is sterilized, a vacuum pulls steam and condensation through the drain vent. The longer the vacuum system runs during the dry phase, the cooler and dryer the goods will be when removed from the chamber.

Gravity Cycles are commonly used on loads like glassware, bio-hazardous waste (autoclave bag waste), and wrapped and unwrapped instruments.

Material Recommended for:

Glassware that must be sterilized upright and/or can trap air

Wrapped dry items that can trap air

Pipette tip boxes

Sharps decontamination

(in collection containers)

Biohazard waste decontamination, in autoclave bags; can be wet or dry

Session # 13 & 14: Tutorial

Marker assisted selection in plant breeding.

The process of developing new crop varieties can take almost 25 years. Now, however, biotechnology has considerably shortened the time to 7-10 years for new crop varieties to be brought to the market. One of the tools which can make it easier and faster for scientists to select plant traits is marker-assisted selection (MAS).

Molecular shortcut

The differences that distinguish one plant from another are encoded in the plant's genetic material, the DNA. DNA is packaged in chromosome pairs (strands of genetic material), one coming from each parent. The genes, which control a plant's characteristics, are located on specific segments of each chromosome. Together, all of a plant's genes make up its genome.

Some traits, like flower color, may be controlled by only one gene. Other more complex characteristics, however, like crop yield or starch content, may be influenced by many genes. Traditionally, plant breeders have selected plants based on their visible or measurable traits, called the phenotype. This process can be difficult, slow, influenced by the environment, and costly – not only in the development itself, but also for the economy, as farmers suffer crop losses.

As a shortcut, plant breeders now use marker-assisted selection (MAS). To help identify specific genes, scientists use what are called molecular or genetic markers. The markers are a string or sequence of nucleic acid which makes up a segment of DNA. The markers are located near the DNA sequence of the desired gene and are transmitted by the standard laws of inheritance from one generation to the next (Figure 1). Since the markers and the genes are close together on the same chromosome, they tend to stay together as each generation of plants is produced. This is called genetic linkage. This linkage helps scientists to predict whether a plant will have a desired gene. If researchers can find the marker for the gene, it means the desired gene itself is present.

GENETIC MARKERS



"The green section indicates the presence of a desirable gene in an organisms' genetic code that is associated with two genetic markers (red flags)."

Source: <http://usda-ars-beaumont.tamu.edu/dblhelix.jpg>

As scientists learn where markers occur on a chromosome, and how close they are to specific genes, they can create a genetic linkage map. Such a map would show the location of markers and genes, and their distance from other known genes. Scientists can produce detailed maps in only one generation of plant breeding.

Using very detailed genetic maps and better knowledge of the molecular structure of a plant's DNA, researchers can analyze only a tiny bit of plant tissue, even from a newly germinated seedling. Once the tissue is analyzed, scientists know whether that seedling contains the

appropriate gene. If it doesn't, they can quickly move on and concentrate on analysis of another seedling, eventually working only with the plants which contain a specific trait.

It should be noted, however, that molecular breeding through MAS is somewhat limited in scope compared to genetic engineering or modification because: 1) it works only for traits already present in a crop; 2) it cannot be used effectively to breed crops which have long generation times (e.g. citrus); and 3) it cannot be used effectively with crops which are clonally propagated because they are sterile or do not breed true (this includes many staples such as yams, bananas, plantain, sweet potato, and cassava).

Molecular Markers

Several marker systems have been developed and are applied to a range of crop species. These are the Restriction Fragment Length Polymorphisms (RFLPs), Random Amplification of Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or microsatellites, and Single Nucleotide Polymorphism (SNPs).

Glossary

AFLP:

Amplified Fragment Length Polymorphism. A highly sensitive method for detecting DNA polymorphism. Following restriction enzyme digestion of DNA, a subset of the DNA fragments is selected for PCR amplification and visualization.

Genetic Map:

A map of the relative positions of genetic loci on a chromosome, determined on the basis of how often the loci are inherited together.

Linkage Map:

A map of relative positions of genes on a chromosome. Genes inherited together are close to each other on the chromosome, and said to be linked.

Microsatellites:

Very short DNA motifs (1-10 base pairs) which occur as tandem repeats at numerous loci throughout the genome. Also known as simple sequence repeats (SSR), simple tandem repeats or simple repetitive sequences.

Monogenic trait (Mendelian trait): a trait determined by the action of a single genetic locus

Nucleic acid:

molecule found in all living cells, in which the hereditary information is stored and from which it can be transferred. The two chief types are DNA (deoxyribonucleic acid), found mainly in cell nuclei, and RNA (ribonucleic acid), found mostly in cytoplasm.

PCR:

Polymerase Chain Reaction. A method for amplifying a DNA sequence in large amounts using a heat-stable polymerase and suitable primers to direct the amplification of the desired region of DNA.

Polymorphism:

A detectable difference at a particular gene or marker occurring among individuals.

RAPD:

Random Amplification of Polymorphic DNA. A widely-used technique for amplifying anonymous stretches of DNA using PCR with arbitrary primers.

RFLP:

Restriction Fragment Length Polymorphism. Variations which occur in the length of DNA fragments produced when DNA is broken down by restriction enzymes (enzymes which recognize specific sequences of DNA, usually 4-6 base pairs long, and cleave the DNA at these points, known as restriction sites).

SNP:

Single Nucleotide Polymorphism. A common, but minute, variation that occurs in DNA sequences of a genome. These variations can be used to track inheritance in families or species.

QTL:

Quantitative Trait Locus. Location of a specific gene that affects a measurable or quantifiable trait. These traits are typically affected by more than one gene, and also by the environment. Examples of quantitative traits are plant height (measured on a ruler) and body weight (measured on a balance)

Quantitative (continuous) traits:

phenotypes that exhibit a range of measurable outcomes.

Session # 15:

Tutorial: Course/Discussion from session 1 to 14 (Mid Term Exam)