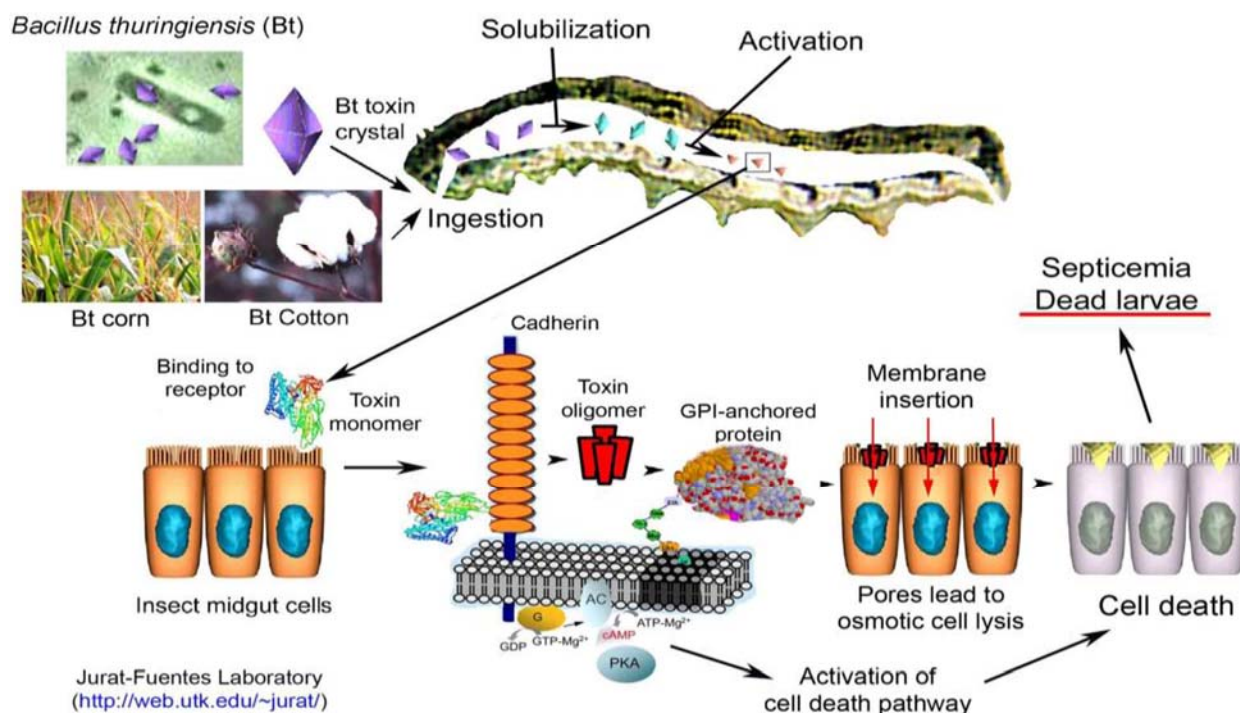


Lecture 8: Plant Biotechnology applications

1- Insect Resistance, Approximately 40 Genes are used in conferring insect resistance in crop plants. , Most popular genes used from microbes in insect resistance are Bt gene from *Bacillus thuringiensis*. Ipt Gene isopentyl transferase from *Bacillus thuringiensis*. Cholesterol oxidase gene from *Streptomyces*. Pht gene from *Photobacterium luminescens*. , Resistance inducing genes from higher plants can be a) Proteinase and amylase inhibitor b) Lectin- snowdrops lectin (GNA) , Resistance gene From animals can be a) Serine proteinase inhibitor

Resistance genes from microorganisms, Bt Toxin gene , Delta endotoxine : works against Lepidopteran larvae but some are specific to Dipterian and Colepteran insect. Protein with approximately 1155 amino acids, More than 100 cry protein sequences have been published , Cry1Ab against European corn borer , Cry1Ac against tobacco budworm and cotton ball worm , Provides resistance against many plant pathogens.



Cholestrol Oxidase(CO): Gene from *Streptomyces* shows acute toxicity to boll weevil larvae in tobacco., Isopentenyl Transferase: a gene from *Bacillus thuringiensis*, codes key enzymes in Cytokinin biosynthesis pathway. Expression in tobacco and tomato has resulted in decrease in consumption. Resistance genes from microorganisms.

2- Resistance genes from higher plants, Non Bt insecticidal gene which produces proteins which interfere with nutritional needs of insect., Proteinase inhibitor: known since 1938 that plants contains peptides acting as protease inhibitor, different proteinase are serine, cysteine, Aspartate and metallo proteinases. Alfa Amylase inhibitor: Gene for amylase inhibitor is isolated from *Phaseolus vulgaris*, enzyme works against *Zabrotes*, *Callosobruchus* inhibits gut amylase, Lectins: Glycoproteins from plants, affects Aphids, and many other sucking and Piercing insects. Works only when ingested in large quantities.

3- Resistance to Abiotic Stresses , Aboitic stresses like Drought , high temperature, low temperature, salinity and alkanity adversely influence growth and induces senecense. , Osmoprotectant lowers the osmatic potential , Common osmoprotectant: Inorganics ions, polyols, soluble carbohydrates, amino acids, Quaternary ammonium compounds such as glycine betaine. , Several enzymes coding enzymes for biosynthesis of these osmoprotectants have been cloned.

4- Fructane biosynthesis , LEAs(late embryogenesis abundant) HVA-1 in barley for rice , HSPs and AtHSF , Fatty acid desaturase for Cold tolerance Resistance to Abiotic Stresses.

5- Herbicide-resistant plants: Reducing the ability of the herbicide - sensitive target to bind to the herbicide , Herbicide: Glyphosate (better known as Roundup) , Resistance to Roundup (an inhibitor of the enzyme EPSP involved in aromatic amino acid biosynthesis) was obtained by finding a mutant version of EPSP from *E. coli* that does not bind Roundup and expressing it in plants (soybean, tobacco, petunia, tomato, potato, and cotton) , 5 -enolpyruvylshikimate-3-phosphate synthase (EPSP) is a chloroplast enzyme in the shikimate pathway and plays a key role in the synthesis.

6- Transgenics for quality , Transgenic for Storage protein , 2s albumin protein gene from brazil nut expressed in tobacco by Phaseolin promoter, , Sunflower albumin protein was not allergic, was expressed in lupin using pea vicilin gene promoter , Amala gene from *Amaranthus* in potato , Soybean gene in rice , beta-casein gene in tobacco , Phytase gene from *Alfa alfa* to tobacco, hydrolyses phytate to inorganic phosphate



Before



After

Eg. RoundUp Ready Canola

The Flavr Savr tomato was a tomato engineered to have a longer shelf life. In 1995, Bt Potato was approved safe by the Environmental Protection Agency.

Bt-Cotton is a genetically modified cotton which is resistant to pests. Golden Rice genetically modified to contain beta-carotene (a source of Vitamin A).

Transgenic fruit obtained from pear and apple. A Blue Rose is a genetically modified Rose.

Fruits and plants: □ In plants, we have blue rose which is a genetically modified rose. □ Bt-cotton is genetically modified cotton which is resistant to pests.

The Flavr Savr tomato was a tomato engineered to have a longer shelf life.

□ In fruits, transgenic fruit obtained from pear and apple.

Golden rice: is genetically modified rice which contains beta carotene (a source of vitamin A)





Golden Rice



Bt Corn

Corn Borer- insect that kills corn crops

European Corn Borer



U.S. + Canada: > \$1 billion per year, damage + control costs



7- Flower and flower colour Role of colour Major plant pigments Genetic improvement of flower colour –Making deliberate crosses between two parents –Mutation –Polyploidy –Genetic Engineering of flower colour. Over-expressing or silencing the structural gene expression in flavonoid biosynthetic pathway.

The global flower industry thrives on novelty. Genetic engineering is providing a valuable means of expanding the floriculture gene pool so promoting the generation of new commercial varieties. Engineered traits are valuable to either the consumer or the producer. The goal of genetic engineering is to improve the characteristics of flowers such as, flower colour, vase life, floral scent, flower morphology, disease as well as pest resistance, flower productivity, timing and synchrony of flowering.

8- Anti-Viral Strategy , TMV-coat protein inserted into tobacco and tomato plant cells using Ti plasmid , Viral capsids inhibit viral replication of TMV when infected ♣ Cucumber mosaic virus (CMV), the most important viruses , more than 800 host plants , absent the resistance genes in the germplasm of most crops ♣ Other trials using capsid proteins: potato leaf-roll virus, cantaloupe mosaic virus, rice strip virus.

Virus resistant papaya- Resistant to papaya ring-spot virus, Transgene- Virus coat protein.

9- Development of stress- and senescence-tolerant plants: genetic engineering of flavorful tomatoes Fruit ripening is a natural aging or senescence process that involves two independent pathways, flavor development and fruit softening. Typically, tomatoes are picked when they are not very ripe (i.e., hard and green) to allow for safe shipping of the fruit. Polygalacturonase is a plant enzyme that degrades pectin in plant cell walls and contribute to fruit softening. In order to allow tomatoes to ripen on the vine and still be hard enough for safe shipping of the fruit, polygalacturonase gene expression was inhibited by introduction of an antisense polygalacturonase gene and created the first commercial genetically engineered plant called the FLAVR SAVR tomato. Flavor development pathway Fruit softening pathway Green Red Hard Soft polygalacturonase antisense polygalacturonase.

10- Modification of plant nutritional content: increasing the vitamin A content of plants , 124 million children worldwide are deficient in vitamin A, which leads to death and blindness , Mammals make vitamin A from β -carotene, a common carotenoid pigment normally found in plant photosynthetic membranes , Here, the idea was to engineer the β -carotene pathway into rice , The transgenic rice is yellow or golden in color and is called “golden rice”

GGPP Phytoene Lycopene β -carotene Vitamin A

Daffodil phytoene synthase gene Bacterial phytoene desaturase gene Daffodil lycopene β -cyclase gene Endogenous human gene

11- Edible Vaccines – Ongoing Research Areas Hepatitis B, Dental caries - Anti-tooth decay Ab (CaroRx™), (anti-*Streptococcus mutans*), Autoimmune diabetes, Cholera, Rabies, HIV, Rhinovirus, Foot and Mouth, Enteritis virus, Malaria, Influenza, Cancer)

12 Frost Resistance , Ice-minus bacteria , Ice nucleation on plant surfaces caused by bacteria that aid in protein-water coalescence forming ice crystals 0°C (32°F) , Ice-minus *Pseudomonas syringae* , Modified by removing genes responsible for crystal formation , Sprayed onto plants , Displaces wild type strains , Protected to 23°F , Dew freezes beyond this point , Extends growth season , First deliberate release experiment – Steven Lindow – 1987- sprayed potatoes , Frost Ban , Different strain of bacteria – Julie Lindemann led different project – 1987 , Strawberries in California



13- Potentially Harmful Effects, Contamination by pesticides, Co-purification of plant chemicals (e.g. nicotine), Different glycosylation in plants versus animals, Interference with normal function of protein in animals, Stimulation of hypersensitivity reactions in animals (allergies) , Research is underway to engineer tobacco to synthesize “human- compatible” glycans

14- Environmental Risks, Pharmaceutical products may inadvertently be introduced into the general food supply , Cross-pollination , Pollen from a drug-containing crop fertilizes a neighboring related crop (or wild relatives) used for animal consumption , Wind , Insects Consumption of GM plant by insects ◊ Food chain , Accumulation in birds – extinction? (e.g. DDT and bald eagle), Deleterious effects on non-target organisms (NTO's) NTO's = organisms in the environment that are affected by the product unintentionally, Insects, arthropods, Risk to NTO's, depends on recombinant protein involved, Risk assessment carried out case-by-case

15- Genetic Engineering in Tobacco plant glows in the dark which has been genetically engineered to express a gene taken from fireflies, in 1986.

16- GM trees are grow faster and easier to break down, with less lignin better to make paper, which could reduce the amount of chemicals and energy used in the papermaking process. Genetic Engineering in Agriculture:

17- GM Corn planted in 2013, tolerance of herbicides glyphosate glufosinate, insect resistance.

18- GM Apple, produced to delay browning, 2015 approved for sale.

19- GM Alfalfa GM sugar beet Tolerance of glyphosate, glufosinate.

20- GM squash, Resistance to watermelon, cucumber yellow mosaic viruses, 2005.

21- GM sugarcane, Pesticide tolerance high sucrose content.

22- Protease inhibitors, Insects have protease in their gut which are the enzymes helping in digestion of protein, PI inhibit the proteases and affect digestion in insects, The PI are isolated from one plant and cloned into another to produce transgenic plants , Four classes- Serine, Thiol, Metallo and Aspartyl , Serine PI is the most important Eg- Cowpea trypsin inhibitor (CpTi) gene derived from cowpea and cloned into tobacco against *Helicoverpa*

23- Alpha amylase inhibitors- Alpha amylase is a digestive enzyme present in insects for digestion of carbohydrates. AAI affects the digestion of carbohydrates in insects. Transgenic tobacco & tomato expressing AAI which are resistant to Lepidopteran Pests Lectin genes- Lectin are a group of plant proteins that bind to carbohydrates, including chitin. The deleterious effect of chitin binding lectins on

24- Prospects of GE of plants for phytoremediation of toxic metals
Phytoremediation—the use of green plants to remove, contain or render harmless environmental pollutants. The use of GE to modify plants for metal uptake, transport & sequestration may open up new avenues for enhancing efficiency of phytoremediation. Metal chelator, biosequestration by plants & prospects of GE
Photosynthetic assimilation of atmospheric carbon dioxide by land plants offers the underpinnings for ‘C’ sequestration. Bioenergy crops serve the dual role of providing biofuel that offsets fossil fuel greenhouse gas(GHC) emissions & sequestering ‘C’ in the soil through root system. ‘C’ captured in plant biomass can also contribute to ‘C’ sequestration.

Genetically Modified organisms (GMOs) Regulations

How are GMO's tested & approved?

EPA- (Environmental Protection Agency) evaluates environmental safety.

USDA- (US Department of Agriculture) evaluates whether plant is safe to grow.

FDA- (Food & Drug Administration) evaluates whether the plant is safe to consume.

LAWS: Current laws allow industries to patent/own the intellectual property that they genetically modify/create, Ownership of the new genome/organism, allows companies to charge whatever fee they wish for use of their patent, including further research in the area.

Advantage & Disadvantage of transgenic plants

Advantages of Transgenic plants, Improvement in nutritional value of food, Increase in farmers income, Increase in food supply, More convenient and flexible to use, Safer environment through decreased use of pesticides, Improved the quality of ground and surface water with less pesticide residues , Safe to non-target organisms and human beings

Disadvantages of transgenic plants , Secondary pest incidence , Disruption of pollinators and plant communities would occur if the toxin is expressed in plant nectar and pollen , CCD- Is affecting bee hives and it is supposed to be the use of Bt transgenic crops , GM ingredients cause cancer- Histopathologist (Dr. Stanley Ewan) “food and water contaminated with GE material could increase the growth of Malignant tumor , GM food could raise new allergy outbreak in humans – GM soybean containing “Brazilian protein” was allergic to humans and was withdrawn from production 40