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on

Biofertilizers

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Pest resistant (Bt-cotton)/Transgenic Plants:

All Bt cotton/soybean/brinjal plants contain one or more foreign inserted genes derived from the soil- inhabiting bacterium, *Bacillus thuringiensis*; thus, they are transgenic plants. The insertion of the genes from *B. thuringiensis* into DNA of cotton plants cells causes cotton plant cells to synthesize crystal insecticidal proteins, often referred to as Cry proteins. These insecticidal proteins are efficient in killing some of the most devastating caterpillar pests of cotton and other crops, such as the larvae of tobacco budworms and bollworms. This new technology for managing insect pests was permitted by the U.S. Environmental Protection Agency (EPA) for commercialization in the United States in October 1995 and is now available from different seed companies in various other cotton-growing countries around the world. Cotton varieties containing the Cry1Ac Bt protein offer protection against three major cotton pests—tobacco budworms, bollworms, and pink bollworms. In addition to above, Bt cotton also effective to manage some other important caterpillar pests such as beet armyworms, cabbage loopers, cotton leaf perforators, fall armyworms, southern armyworms, and soybean loopers. Bt cotton effectively manage to the tobacco budworms, pink bollworms, and European corn borers is as compared to the others available effective foliar insecticides.

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The insect-parasite disease-causing microorganism *Bacillus thuringiensis* (Bt) is a naturally occurring soil borne bacterium found across the globe. Nature have given a unique feature to *Bacillus thuringiensis* to synthesize crystal-like proteins that have peculiarity to kill selectively of definite groups of insects. When the insect eats Cry proteins released by the colonies of *Bacillus thuringiensis*, during the feed, digestive enzymes of cry proteins activate the toxic nature of cry protein. Cry proteins fix to specific receptors on the intestinal walls and rupture midgut cells of the insect. Once the susceptible insects eaten small or enough amount of toxic cry protein (60 types), expire within 2 or 3 days. A wide range of cry proteins synthesize by different strains *Bacillus thuringiensis* (Bt), and there are more than hundreds of known strains. Most Cry-proteins are active against specific groups of insects, such as, Cry3A proteins is very effective against colorado potato beetle larvae; Cry1Ac is potent against tobacco budworms; and Cry1Ab, Cry1F, Cry1Ac, and Cry9c proteins is enable to manage the European corn borers.

Protein gene.

Molecular inserted package in Bt Cotton:

Bt protein:

A segment of gene, isolated from *Bacillus thuringiensis*, is known to synthesize cry protein is called "Bt gene". Bt gene inserted into cotton plant cell to express the gene to synthesize the cry protein. The Bt gene, modified for new expression in cotton, enables the cotton plant to synthesize Cry-protein. The first varieties of Bt cotton developed in the United States enabled to synthesize one Cry-protein gene—Cry1Ac. Another developed variety contain a "stacked" gene complex, of which one gene act to manage insect (Cry1Ac) while another one is known to enhance the ability of tolerance of cotton crops against herbicide glyphosate to use the weed control.

Promoter:

A promoter is a DNA fragment that regulates the amount of Cry-protein synthesis in desired portion of plants. Some promoters restrict protein synthesis to specific parts of the plant, such as leaves, green tissue, or pollen while some other promoters are known to synthesize Cry-protein throughout the body of plant including Bt cotton and certain Bt corn varieties. Promoters play a vital role in swich on and switch off the function of protein synthesis. Maximum Bt cotton transgenic crop available in market is known to synthesize Bt protein throughout the growing season.

Genetic Marker:

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A genetic marker enables to breeders and molecular biologist to detection and confirmation of successful insertion of a gene into the DAN of plant cell through use of cloning and vectors. It also helps plant breeders in identifying and developing new improved cotton lines with the Bt gene. A common marker is an herbicide tolerant gene linked to the Bt gene. Interstation of Bt and marker gene into the DNA of plant cell would successfully survive after herbicides treatment while only Bt gene carrying plants will be killed. It means that linking of Bt gene with marker gene and their indentation in DNA of plants cell is essential to make the plant the herbicide resistant. Molecular package—a Bt gene along with a promoter and marker should be inserted into DNA of cotton plant through a variety of plant transformation tools and techniques. Characteristic of transgenic plants may be affected by the genetic package, and their location of the inserted genes in the plant DNA which would influence the synthesis of Bt protein and other key functions of plant as well. Keeping these constraints in view, transgenic seed producing companies carefully scrutinize their transgenic seeds very precisely to ensure adequate synthesis of Bt protein (cry protein).

A transgenic Bt cotton plant synthesizes cry-protein within the plant tissues that caterpillars eat overcomes most of the concern biotic constraints. Synthesized cry protein in the cells of plant is durable and sustainable without degradation, since they are not directly in contact of ultra violet rays, irradiation, rain or wined etc. Cry protein is always persisting in plant tissues whenever newly hatched larvae feed its first bite will dead 2-3 days after

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Herbicide resistant plants (RoundUp Ready soybean):

Roundup Ready Soybean is a genetically engineered crop developed by insertion of a foreign designed DNA which have resistance/tolerance for herbicide glyphosate.

Roundup (Glyphosate) herbicide:

Roundup is a tradename of product of Glyphosate molecule which is widely used as herbicide or weedicides. In 1970, glyphosate is synthesized by John E Franz of Monsanto Company as a non-selective herbicide. The Monsanto was an American Agrochemical and Seed based company. Monsanto developed a glyphosate molecule used as herbicide which tradename is Roundup, that quickly became the most commonly used herbicide in the world. When Glyphosate or Roundup (4ml/litre) sprayed on foliage of herbs and plants, molecules is absorbed by the green foliage of herbs/plants and the molecules moves from there to the apical part of roots and shoots of the plant. After reaching at the apical portion, glyphosate start to inhibit the synthesis and activities of enzyme 5-enolpyruvylshikimat3-phosphate synthase (EPSPS). EPSPS enzyme is essential in shikimate pathway for biosynthesis of aromatic amino acids viz phenylalanine, tyrosine and tryptophan which are essential for plants, animals and humans to execute the physiological and biochemical functions. Plant and microorganism are only the source of EPSPS enzyme to meet the requirement of it in form of foods by animals and humans. Glyphosate has the analogous chemical structure with phosphoenolpyruvate (PEP), and act as strong competitive inhibitor for phosphoenolpyruvate (PEP), it would easily react with the enzyme EPSPS. Indeed, Glyphosate (substrate) can bind to react on active site of the enzyme EPSPS more tightly than the phosphoenolpyruvate (PEP) substrate. Since, Glyphosate is a pseudo-substrate of enzyme EPSPS, so it would close the function of shikimate pathway, as a result, the synthesis of aromatic essential amino acids viz. phenylalanine, tyrosine and tryptophan will be declined. Eventually, the plant dies due the deficiency of aromatic amino acids. Farmers uses the Roundup (Glyphosate) as herbicides to kill the collateral plants grown as weed without giving any adverse impact on economically valuable plants withstand. Since Roundup, the

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glyphosate-based herbicide, kills every plant, farmers needed a way for their plants to withstand the effects of the herbicide.

Scientific Discovery of Roundup Ready Soybean Crops:

In the 1980s, aroA gene has been identified in CP4 strain of *Agrobacterium tumefaciens* which is act as glyphosate resistant mutant EPSPS enzyme. aroA gene has been isolated from the CP4 strain of *A. tumefaciens* by Scientist of Monsanto. Monsanto developed the first genetically engineered plant seeds by inserting the aroA gene in DNA of Soybean crops to develop the tolerance against glyphosate herbicide that might withstand Roundup and called them Roundup Ready Crops.

Aro denotes genes that regulate the biosynthesis of aromatic amino acids viz. phenylalanine, tyrosine and tryptophan and **A** stands for a mutation type of the gene in transgenic soybean Roundup Ready Crops. The glyphosate tolerant form of the EPSPS enzyme is called CP4 EPSPS enzyme. CP4 EPSPS enzyme has a played vital role in declining the binding affinity for glyphosate (substrate) with EPSPS enzyme, which means that CP4 EPSPS enzymes binds to the endogenous PEP substrate of Roundup Ready Crops by avoiding to bind and react with competitive inhibitor glyphosate. Roundup Ready Crops would survive upto harvest after spraying of herbicide (glyphosate) because Roundup Ready Crops would enable to synthesize essential amino acids viz. phenylalanine, tyrosine and tryptophan by active function of shikimate pathway. Seeds of Roundup Ready Soybean Crops has given a great remedy and relief to the farmers to soybean with easy management of weeds.

Insertion of aroA gene isolated from CP4 strain of *Agrobacterium tumefaciens* in soybean crops to develop Roundup Ready Soybean Crops:

To develop round ready soybean crop, the isolated aroA gene from CP4 strain of *Agrobacterium tumefaciens* can be introduced into soybean using various genetic engineering and molecular tools and technique. In this technique, seeds of soybean should grow on prepared growth medium by using desired nutrients in petri plate under tissue culture lab. After germination, when the seeds attain growth upto cotyledon stage, a fine cut should make over the cotyledon with sterilized blade and some drops of spore suspension of *Agrobacterium tumefaciens* can be pour in the cut made on cotyledon under aseptic conditions and shift the cotyledons containing petri plate in growth chamber. When

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Agrobacterium tumefaciens, reached in wounded tissue of cotyledon through the cut, tissues synthesize the phenolic compounds to attract the *Agrobacterium tumefaciens* and provide avenue to transfer the DNA of *Agrobacterium tumefaciens* into cells of soybean plant. After entering the DNA of *Agrobacterium tumefaciens* in to the cells of soybean will start to integrate with DNA of Soybean to make the Roundup ready soybean plant. At few days later, cotyledons should transfer/placed in a glyphosate containing medium to observe the tolerance developed in Soybean plant against the glyphosate. If the plant has potential to exhibit the tolerance, apical growth will be continued and a successful Roundup ready soybean plant is developed.

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