GENETICALLY MODIFIED CROPS & THEIR ADVANTAGES OVER TRADITIONAL CROPS

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Abstract: Genetically Engineering have been used from a long time to get the desired traits in the produce for many reasons either to achieve high yield or to resist the stress conditions. Throughout history many crops have been modified from their original state by selection, controlled breeding etc. With time, new methods have been developed to modify crops genetically using genetic engineering approach, to develop new plants with different characteristics more rapidly with best results like high yield with good quality, insect resistance, drought & stress tolerance and Phytoremediation etc. Genetically Modified Crops were in a huge debate for its use and their effects on human and environment. But with modern methods and more safer approach GM crops have the potential to solve world's hunger and malnutrition like big problems and to contribute to the ecosystem by reducing the use of chemicals, fertilizers and pesticides. This review will address different methodologies use to develop the Genetically Modified Crops from history times to present, Ways to identify the presence of GMOs, Present GM Crops with their modified traits.

Keywords: Genetically Modified Crops, Genetic Engineering, GMOs, Phytoremediation.

I. INTRODUCTION

Genetically modified crops are plants used in agriculture in which the DNA has been modified using genetic engineering approach to get the desired quality. The aim is to introduce a new trait to the plant which does not exist naturally in the plant. The resulting modified plants are called "GM Crops" or "Biotech Crops". Example: - in non-food crop, include production of biofuel or other industrial useful good. (ISAAA, 2013) Genetic Engineering or Genetic Modification refers to alteration of DNA in an organism to change its characteristics in a particular way. History of Genetic Engineering started way back 10,000 years ago when farmers started selective and cross breeding approach to obtain the best desired traits in the plant. In 1992 the first genetically modified food crop, FLAVR SAVR tomatoes were made available. (NCBI, 2004) In today's time there are many genetically modified crops available which include: sweet and field corn, soybeans, cotton, canola, alfalfa, sugar beets, papaya, potatoes, and squash etc.

1983- First time, scientists created a genetically modified tobacco resistant to an antibiotic (Lemaux PG, 2008)
1985- First GM crop trials begin worldwide
1987- Bt tobacco was tried on field first time
1993- USFDA allows companies to market GM seed
1994- First GM tomato, the Flavr Savr, got approval in US for selling in market. (Zadoks JC, 2000)
1996- Genetically Modified soya bean with Herbicide-tolerant become available in US (Dill GM, 2005)
2000- Golden Rice was engineered for overcoming the Vitamin A deficiency worldwide. (Ye X, 2000)
2004- UK Government approved Genetically modified maize for commercial production. (Burke D,2004)
2011- GM crops were grown by farmers worldwide in 29 countries on 160 million hectares of land. (Kush GS, 2012)
2016- 185.1 million hectares of land were planted with GM crops by 18 million farmers. (ISAAA, 2016)
2017- Pursued work for betterment of biotech crops. (Kolbe AR, 2017,Mishra A, 2017)

Table 1: History of Genetic Modifications in crops

II. Methodologies for the development of genetically engineered plants:

***Non Genetic Approaches*:**

a) Simple Selection

Simple Selection is the simplest method to modify the plant genetically. In this method, crop is supervised, and selection is made on basis of traits expressed in plant and its performance. Plants with desired traits are selected from the crop and use to produce the new generation for the continuous proliferation. Rest breed or crop is utilized for eating or feeding animals. And seeds from the desired plants are collected and saved for plantation for next time.

Gradually this method of selection has been improved with modern technology of Simple Selection- "marker assisted selection". It is an indirect method in which selection of gene of interest is based on marker linked to it and molecular analysis is done for detecting the expressed traits in plant population (Ribaut, 1998). With this method, identification of the plant carrying most desired traits becomes easy and fast.

b) Cross-Breeding

It is a method of interbreeding two closely related plants to produce new variety of crop, to produce the desired traits within the plant or to combine the useful characteristics of two plants. This is done by migration or bushing of pollen of one plant onto the stigma of another plant and resulting plant is the hybrid of them carrying the traits of both plants. This process is also known as "Cross Pollination". Then, plant breeders select the plants with most beneficial traits and use them for the next generation.

Crossbreeding can only make use of desirable traits if they are in the same or closely-related species of plant, therefore additional techniques have been developed to create new traits for plant breeders to use.

c) Mutagenesis

Mutagenesis or Mutation Breeding is the process where the genetic information of an organism is changed either in a stable, heritable manner, or in nature or induced experimentally. This method makes use of mutagens or mutagenic agents like ethyl methanesulfonate or radioactivity to generate random changes in plants by altering the DNA sequences of the plant. Plants created by using mutagenesis are called mutagenic plants.

The dose of mutagenic agent used for the mutagenesis can be adjusted by breeder to achieve thedesired traits as result. In agriculture, these genetic changes are used to improve the crop's useful traits that occurs naturally.Typically,many plants or seeds are mutagenized, which canbe grown to their reproductive maturity, then useful mutations are identified by breeders and progeny are derived.

d) Protoplast fusion

Combination of sperm cells in pollen with the ova in the ovaries of a flower, known as fusion of two cells into one.Doing it artificially in laboratoryis called Protoplast fusion or Somatic Fusion or Somatic Hybridization. In this method, the useful traits can be transferred from one species to another by fusing the cells or cell components. If the fusion occurs successfully and the resulting protoplast hybrid is compatible and healthy, may results into a hybrid plant that will carry the genetic material or traits of both species. Hybrids can be produced either from different varieties of the same species or from two different species.

e) Polyploidy

It is defined as the occurrence of more than 2 sets of chromosomes. It is commonly found in plants andmay occur due to abnormal cell division, meiotic or mitotic failures, and fusion of unreduced (2n) gametes.

It can also be induced in plants and cell cultures by using some chemicals like colchicine, which can result in chromosome doubling. Polyploidy in crop plants is most commonly induced by treating seeds with colchicine. This technique is usually used to increase the size of fruits or to make the crop sterile, to enhance the colour of fruit.

f) Somaclonal Variation

Somaclonal variation is the changes occur spontaneously during tissue culture of higher plants (when plant cells are grown in-vitro). Mainly, changes occurs in chromosome numbers, chromosome structure and DNA sequence in this method. During the variation, both qualitative and quantitative traits are affected. Resulting variety is neither analysed at that time by the breeders and nor controlled by developers. Plant/crop improvement is the prime advantage of this method.

g) Embryo Rescue

In some plants, cross-pollination is done and their resulting fertilized hybrid embryo also develops but this embryo is unable to mature and sprout. To overcome this problem, embryo is secured after developing. In this, pollination of plants done naturally but embryo is removed before it stops growing and place in a tissue-culture environment to complete its development and sprouting. This technique is used to transfer genesfrom distant, sexually incompatible relatives through intermediate, partially compatible relatives of both the donor and recipient species.

Genetic Engineering Approaches:

a) Agrobacterium tumefaciens

*Agrobacterium tumefaciens*is a naturally occurring gram-negative soil bacterium that can incorporate a portion of a plasmid DNA into plant cellsat a semi-random location and causescrown gall disease. (NCBI, 2004) Because of this unique property of transferring DNA, scientist used it to develop a new strain of Agrobacterium that lacked the disease-causing genes but carry the ability to attach to susceptible plant cells and transfer DNA. The unique mode of action of *A. tumefaciens* has enabled this bacterium to be used as a tool in plant breeding and it is now responsible for majority of Genetic Engineered plants in commercial production (Duan K, 2017).

The Agrobacterium-mediated transformation process:

(a) Genes of interest is isolated from the source organism.
(b) Transgenic construct is matured by promoters; codon; gene of interest; and marker genes
(c) Transgene is inserted into the Ti-plasmid;
(d) Plasmid containing gene of interest is introduced into Agrobacterium;
(e) Transformed Agrobacterium with plant cells can grow together for the transfer of T-DNA into plant chromosome;
(f) Transformed cells are regenerated into genetically modified (GM) plants; and
(g) Trait performance in plant cell is tested at different platforms like test performed at laboratory, greenhouse and fields.

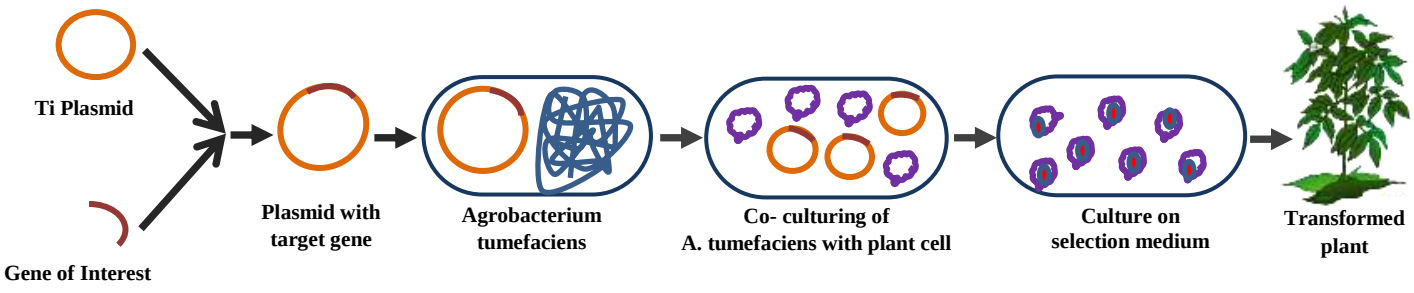

Figure 1: Process of Agrobacterium -mediated transformation

The advantages of using Agrobacterium-mediated transformation over other transformation methods are: Reduction in transgene copy number, and Intact & stable integration of the transgene (newly introduced gene) into the plant genome (Jones et al., 2005).

b) Particle Bombardment

Particle Bombardment is the technique in which foreign DNA is transferred into plant cells or tissue at a very high speed by "shooting" them with a microscopic pellets or gene gun (Sanford JC, 1990). The techniquewas developed by Professor Stanford and co-workers of Cornell University (USA) in 1987. It is also known as particle gun method, biolistic process, microprojectile bombardment or particle acceleration.

The particle bombardment method starts with coating tungsten or gold particles with plasmid DNA. The DNA coated particles are coated on a macro-projectile, which is shot into plant tissue on a petri plate with air pressure (Gan, 1989). Today the gene gun is used for genetic transformation of many organisms to introduce a diverse range of desirable traits.

Plant transformation using particle bombardment follows the same outline as Agrobacterium-mediated method. The steps taken include:

1) Gene of interest is isolated from the source organism;
2) Functional transgene is developed which contains a promoter; a codon, gene of interest; and marker genes.
3) This transgene is then incorporation into plasmid;
4) Transgenes is introduced into the plant cells;
5) Plant cell is allowed to regenerate.
6) Trait performance in plant cell is tested at different platforms like test performed on fields and laboratories.

Particle bombardment also plays an important role in the transformation of organelles such as chloroplasts, which enables engineering of organelle-encoded herbicide or pesticide resistances in crop plants and to study photosynthetic processes.

This technique is most suitable for those plants which are difficult to regenerate and do not show satisfactory or desired response to gene transfer through Agrobacterium for example, rice, wheat corn, sorghum, chickpea and pigeon-pea.

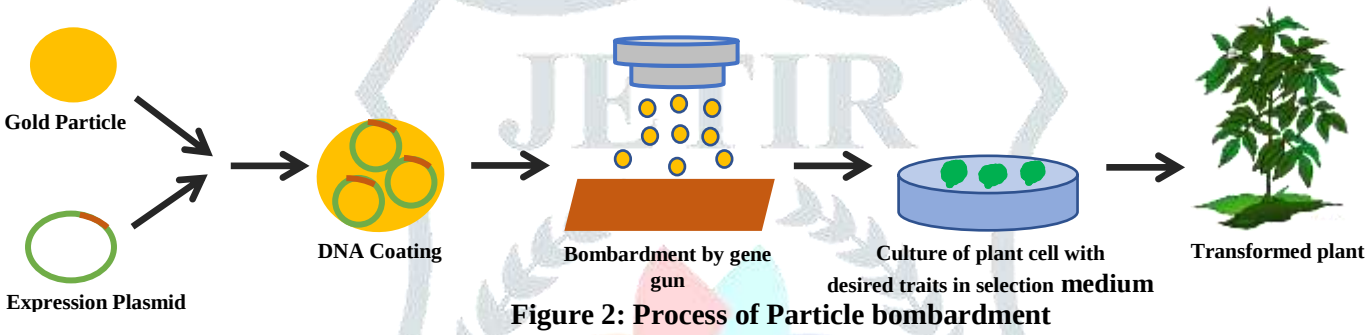

Figure 2: Process of Particle bombardment

c) Microinjection

Microinjection is the process of injecting transgene at microscopic level manually by using a fine glass micropipette. The transgene, in the form of plasmids, cosmids, phage, YACs, or PCR products, can be circular or linear and need not be physically linked for injection.

The steps involve in the process are:

1) DNA is inserted directly into the nucleus mechanically using a glass micropipette injection.
2) Cells is held by suction tube and protoplasts are immobilized in low melting agar, while working under a microscope.
3) DNA is then directly injected into the nucleus.
4) The injected cells are then cultured in-vitro and regenerated into plants.

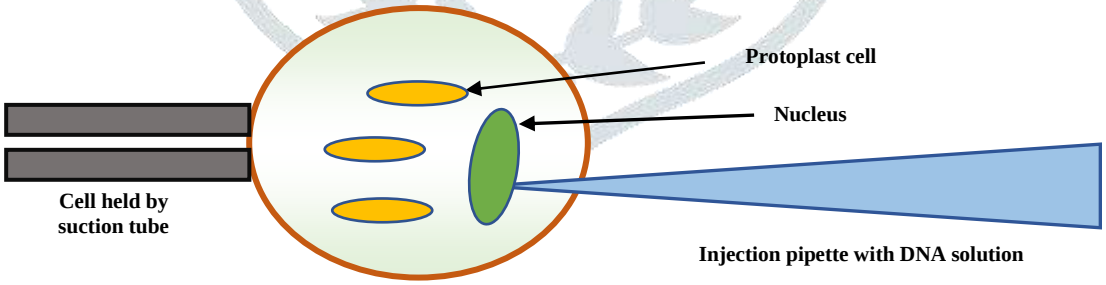

Figure 3: Illustration of Microinjection technique

The two types of microinjection systems used are -

1) Constant flow system

In this system, the amount of sample injected in the cell is determined by the duration of needle remains in the cell. This system is relatively simple and low-cost system (DeanD.A, 2011).

2) Pulsed flow system

The systemhas greater control over needle placement in the cell, volume of substance injected in the cell and has better precision (Dean D.A, 2006). The components of thissystemare expensive but this technique results in less damage to the cell receiving material.

Microscopes used for this technique are - traditional compound microscope, dissecting stereomicroscopeand inverted microscope. Successful examples of this technique are- rapeseed, tobacco, etc.

d) Electroporation

Electroporation, or electro-permeabilization is a technique in which electric field is applied to plant cells to temporarily destabilize the cell membrane and allowing DNA to be inserted in the cell. Plant cell electroporation generally utilizes the protoplast because thick plant cell walls restrict the macromolecule movement. (Bates, 1999)

Electroporation have two systems-

1. Low voltage and long pulse method: 300- 400 V/cm for 10-50 milliseconds.

2. high volt and short pulse method: 1000- 1500 V/cm for 10 microseconds (more stable).
Electroporation is a good choice, as the method is very effective, less costly, fast, and high results can be produced.
This method has been used to produce plants of several species like, rice, wheat tobacco, maize, etc.

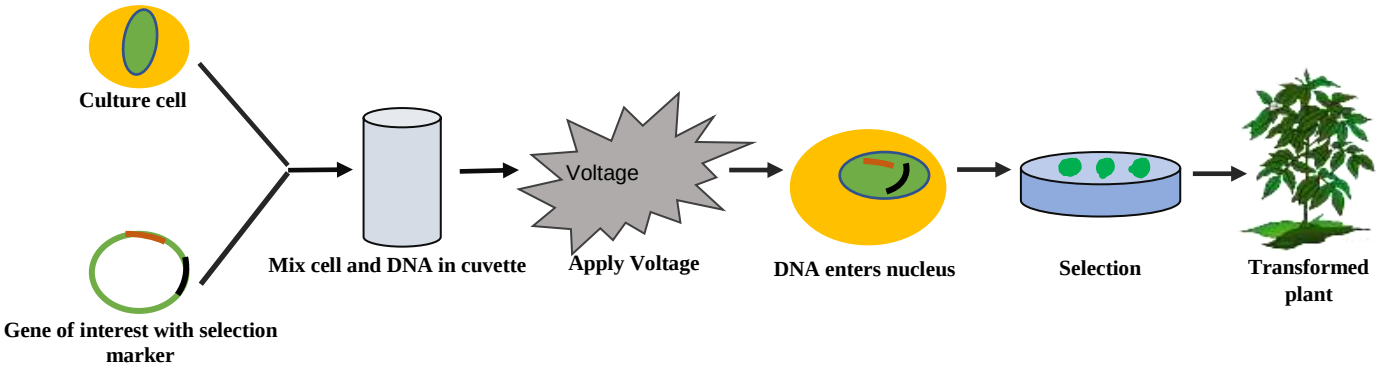

Figure 4: Illustration of Electroporation Method

III. METHODS:

Methods for the detection of transgene in GMOs in food obtained from genetically engineered crops**:**

a) DNA based methods

- ***Southern blotting***

In Southern blotting, transfer of DNA molecules, usually restriction fragments, from an electrophoresis gel to a membrane support like nitrocellulose or nylon sheet takes place in such a way that the DNA banding pattern present in the gel is reproduced on the membrane (Southern E, 2015). The technique involves separation, transfer, hybridization of molecules. Southern blots allow to determine the molecular weight of a restriction fragment and to measure the relative amounts in different samples.
The Southern blot method is used to detect the presence of a particular piece of DNA in a sample. The DNA detected can be a single gene, or it can be part of a larger piece of DNA
In regards to, genetically modified organisms, Southern blotting is used as ultimate test to ensure that genome of the host organism has been successfully incorporated by the particular section of DNA of known genetic sequence.

- ***PCR***

The PCR technique is a highly sensitive and specific GMO detection technique in which DNA is amplified billions of times in order to make it possible to detect and quantified. In this specificity for DNA to be inserted is practised by making primers (short pieces of DNA) which are complementary to the transgenic DNA sequence.
Amplification is accomplished by using DNA polymerase enzyme, which catalyses DNA synthesis, using primers as templates for synthesis of new DNA strand. Primers are specific to transgenic DNA sequence, they bind to an area of the DNA near or within the sequence of GMO such that only this area of the DNA genome is amplified during the process or reaction.
There are various ways to detect GM DNA in a sample are foreign DNA sequence, Broad-spectrum GMO tests, Event-specific and construct-specific GMO tests, Combination of broad-spectrum and specific GMO tests.
The PCR method can detect all commercialized GMOs. The technique is effective with a broad variations of sample types (seed, grain, processed ingredients, finished products), and can provide precise quantification of GMOs as analysis is performed directly at the DNA level. For all these reasons, PCR is the standard GMO test method used in food industry (Salisu I.B, 2017).

b) Protein based methods

- ***Western blotting***

In this technique, a mixture of proteins is separated based on their molecular weight and type, through gel electrophoresis. Results are then transferred to a membrane producing a band for each protein. The membrane is then incubated with labels antibodies which are specific to the protein of interest. The unbound antibody is washed off while bound antibodies are left behind to bind with the protein of interest. The bound antibodies are then detected by developing the film which should show only one band because of bounding between antibodies and protein of interest. (Mahmood T, 2012)
The western blot is a highly specific method that provides qualitative results suitable for determining whether a sample contains the target protein below or above a pre-determined threshold level (Lipton, 2000), and is particularly useful for the analysis of insoluble protein (Bratt, 1999).

- ***ELISA***

ELISA (Enzyme-linked immunosorbent assay) is a sensitive antibody-based GMO detection method in which a particular GMO protein sample is added to a multi-well solid plate on which GMO protein specific antibody has been immobilized (Ahmed F.E, 2002).If the GMO protein is present in the sample it will bind to the immobilized capture antibody. After washing, a different antibody, also specific for the protein of interest and tagged with an enzyme, is added to the well. The enzyme linked detection antibody will bind any GMO protein already immobilized to the well by the capture antibody. After another round of washing to remove any unbound antibody, the substrate for the enzyme is added which induces a color change in the solution. The degree of color change is directly proportional to the amount of GMO protein present in the well.
ELISA tests are not commonly used for GMO detection due to the need for intact protein, a laboratory setting, and the fact that genetic analysis provides equivalent or greater sensitivity.

GENETICALLY ENGINEERED CROPS WITH TRAITS

Table 2: Crops listed with their sources and traits developed in modified plants.
List based on ISAAA- GM approval database
(http://www.isaaa.org/gmapprovaldatabase/cropslist/default.asp)

Crops	Source of Inserted Traits	Traits developed in plant	ISAAA (Event name)
Maize	Bacteria, other species of corn	Resistance to insects	32138, 3272, 59122, 4114, 33121, 678, BT10
		Tolerance to herbicides	
		Male corn sterility	
		Reduction of yield loss under water-limited conditions	
Cotton	Bacteria	Tolerance to herbicides	3006-210-23 x 281-24-236 x MON1445
		Resistance to insects	
Soybean	Bacteria, corn, oats, other species of soybean	Tolerance to herbicides	A2704-12, DP305423, DAS81419 & IND-ØØ41Ø-5
		High oleic acid soybean oil	
		Resistance to insects	
		Tolerance to drought stress	
Melon	Bacteria	Resistance to antibiotics	Melon A, Melon B
		Delayed ripening	
Potato	Bacteria	Resistance to insects	1210 amk, AM04-1020, BT06, BT10, BT12, E12, EH92-527-1
	Potato virus	Resistance to potato virus	
	Other species of potato	Lower level of reducing sugars	
		Lower level of free asparagine	
		Reduced black spot bruising	
Tomato	Bacteria	Delayed softening	1345-4, FLAVR SAVR™
		Resistance to insects	
Eggplant	Bacteria	Resistance to insects	BtBrinjal Event EE1
		Resistance to antibiotics	
Alfalfa	Bacteria	Tolerance to herbicides	J101, J163, KK179
Sugar beet	Bacteria	Tolerance to herbicides	GTSB77 (T9100152)
Rice	Bacteria	Tolerance to herbicides	LLRICE06, LLRICE62
Apple	Other species of apple	Reduced browning and bruising	GD743, GS784 & NF872
Squash	Viruses	Resistance to viruses	CZW3 & ZW20
Papaya	Viruses	Resistance to viruses	55-1
Flax	Mustard green	Tolerance to herbicides	FP967 (CDC Triffid)
Plum	Viruses	Resistance to viruses	ARS-PLMC5-6
Wheat	Bacteria	Tolerance to herbicides	MON71800
Creeping Bentgrass	Bacteria	Tolerance to herbicides	ASR368

IV. COMPARISON

Genetically modified crops as better substitutes in comparison to Traditional crops. There are many advantages of GM crops over traditional crops:

a) Pest & Disease Resistance

One of the most important benefits of Genetically Modified seeds/crop is their potential to resist pests and diseases. Benefit of plant resistance leads to healthy crop and helps to reduce the amount of chemicals used for plants to protect them from pests & diseases. And thus, can reduce pollution introduced in environment by use of chemicals. Ex- BT cotton have resistance against bollworm, beetles etc.

b) Herbicide tolerance

Another benefit of Genetically Modified crop is to show resistance against weeds that demands time and money both of farmers for getting rid of. Crops modified genetically proved to be resistant against powerful herbicides and helps prevent environment damage causes by use of herbicides. Ex- Glyphosate-tolerant soybean.

c) Drought tolerance

Genetically Modified plants have better chance at survival in harsh weather conditions like drought. Crops are engineered to withstand weather extremes and fluctuations, which means that there will be good quality and sufficient yields even under a poor or severe weather condition. Ex- Resistance of rice against critical water conditions by the change in gene Arabidopsis HARDY (effect of increasing the water efficiency of rice by increasing the rate of photosynthesis, and decreasing the amount of water loss through transpiration) (Karaba A, 2007).

d) Improve Nutrition

Genetic Modified crops plays an important role in developing countries where nutrition is most concern. Genetic Modification provides potential to improve the nutrition value of crops in terms of vitamin or mineral content. This helps people get the nutrients they need, and plays a significant role in fighting against malnutrition. For Ex- Golden Rice with presence of Vitamin A can withstand against problem of malnutrition across the world (Key S, 2008)

e) Pharmaceutical

Genetic Modified crops has the potential to provide edible plant vaccines that could be used to immunize individuals against a wide variety of infectious diseases ranging from cholera to potentially AIDS (Moffat A.S, 1995). Ex- Glycoprotein gene gp 120 of the AIDS virus HIV-1 incorporated into GM maize as a cheap edible oral vaccine.

f) Phytoremediation

Soil and water pollution continues to be problem in all parts of world. Plants like popular tree can be genetically modified to remediate soil and water by cleaning up heavy metals or contaminants (Reichenauer T.G, 2008).Ex-Grey poplar trees with overexpress γ-ECS (from *Escherichia coli*)has an elevated capacity for phyto-chelatine production and detoxification of organic pollutants.

V. CONCLUSION

With evolution, methods of modifying plants/crops have also been evolved. Genetic Engineering becomes more desirable approach for altering the crops as it provides various advantages like high yield, resistance to diseases, etc. GM is basically becoming the solution to the growing problems of hunger and malnutrition in the world. Also with the increasing demands for safe and more efficient agriculture, particularly in the rapid changes in climate and Earth's population, it is crucial to adopt scientific approach to develop solutions like GMOs.

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