

Genetically modified crops and their biosafety concerns

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Abstract: Genetic engineering (GE) brings the revolution in crop improvement by developing the genetically modified (GM) crops having intentional and novel traits. GM crops hold the great potential to face current challenges, in term of satisficing the increasing demand of agricultural products and food security. Despite the promises they hold, safety assessment of the GM crops is inevitable for their adoption and public concerns. Intense safety research work has been done, which indicates no direct significant adverse effect either on environment or on human health. However, in spite of intense scientific research work and available information some stones still need to be unturned. A deliberate scientific effort is required to uncover many secrets such as, mutagenicity and long-term health effect of GM crops, in order to build enough confidence for the acceptance of such type of biotechnological innovations.

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Introduction

Genetically modified organism (GMO) is an organism, whose genome is purposely altered in a laboratory through GE technology for introducing new genes or silencing the existing ones, to introduce the desired and novel traits. (Stemke, 2004). It is essential to trace the history of GMOs to fully understand the promises they hold and their impact on human life, like how GE technology enables us to manipulate the genetic material of an organism and how its foundation has laid. GMOs are not new their history can be traced as far back as dawn of civilization, continuous effort has been documented to improve the organism's (plant and animal) yield (Drewes, 1993). The selective breeding (artificial selection) is the primitive method used by human after discovery of Mendel's genetics in the 1860, (SEMINAR, 2014) to enhance the organism's productivity. In selective breeding, the organism with desired qualities was chosen and bred to produced offspring with that desired and novel traits for many generations (Kimmelman, 2013). Although selective breeding is quite promising to improve organism's efficiency but it has many limitations and drawbacks. In conventional breeding method organism share large, unregulated fragments of their genomic DNA. That event can result in transfer of both desired and undesired traits in the offspring. These undesired traits sometime can cause hazards e.g. a conventionally bred potato variety produced excess amount of

glycoalkaloids. That cause alkaloid poisoning which results in gastrointestinal, neurological, circulatory, and dermatological problems (Roots, 2007). To develop an organism with stable desired trait, breeders have to bred the organism many times over multiple generations That extensive crossbreeding laid to many complications, like introduction of unwanted genes, extensive management and high cost. The main limitation of selective breeding is the exchange of genomic material occurs only in organisms who belong to same species (Zohary et al., 2012). These problems were overcome by the discovery of DNA in mid-20 century, which brings the revolution in biology that compel scientist to exploit the organism traits by manipulating the DNA in laboratory know as genetic engineering (GE). GE technology allow to insert only target gene and also avoiding the extensive crossbreeding over multiple generation for introducing the desired traits. The biggest milestone in GE technology is the transfer of desired genes across the species to acquire the novel traits(Woolsey, 2013). **Table 1** contains the mainly developed GMOs with their novel products.

Genetically Modified Crops In Agriculture

GM crops revolutionized the agriculture by improving the crops productivity to ensure an adequate food supply, better nutritional quality, taste, tolerance to herbicide, resistance to certain pests and diseases, longer shelf life and facing the non-biological stress (Meiri and Altman, 1998). Now GM

crops are widely practice across the globe due to the potential they hold like economically beneficial, novelty of products and its ability to fulfill the demand of food supply of the increasing population. Conventional agricultural practices have serious limitation and drawbacks. It cannot satisfy the food

and product demands of world’s increasing population in an economic way (James, 2002). In 1980’s GE technology were extensively used in crop improvement. In 1983, first GM plant (*Nicotiana tabacum*) was developed who have ability to resist the antibiotics (Woolsey, 2013).

Table 1. Mostly developed GMOs and their novel products

Bacteria	Transgenic plants	Transgenic Animal	Yeast or Fungi
Antibiotics	Human lysosomic enzymes	α -Antitypic	Beverages
Insulin	Human glucocerebrosidase	Avidin	Vaccines
nterleukins2 and 3	dismutase α , γ - <i>Bacillus thuringiensis</i> Proteins	Superoxide	dismutase Streptokinase
Interferon		Lymphotoxin	
Vitamin C	Aprotinin	Epidermal growth factor	Hirudin
Bacterial vaccine	Vaccines	Human serum albumin	Aprotinin
Amino acids	Pesticides, Viral, Herbicide resistance	Calcitonin	γ -Interferon
Bioremediation		Tissue cells	Interleukins 3
Indigo-chemicals		Fibrinogen, Antithrombin	collagen Industrial
Enzymes	Insecticides		Enzymes HIV-1 antigens
Proteins (Bt) N and H fixation			

Table 2. GM crops and their related traits

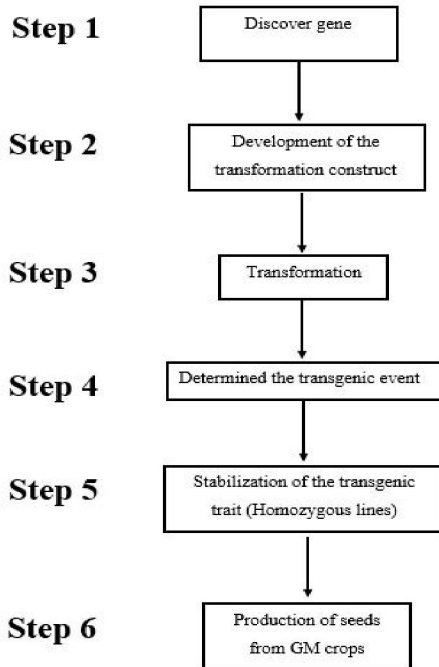
Traits	GM crops
Insect resistance	Cotton, tomato, potato, maize
Herbicide resistance	Maize, rice, cotton, canola, chicory, soybean, flax, linseed, tobacco
Male sterility	Canola
Fertility restoration	Canola, chicory, maize
Delayed ripening	Melon, tomato
Viral resistance	Papaya, squash, potato
Oil modification	Canola, soybean

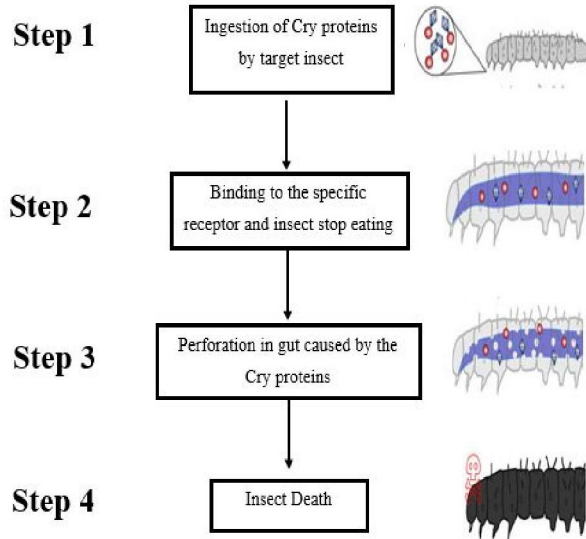
Insect resistance is mostly accomplished by the introducing the *Bacillus thuringiensis* (Bt) coding sequences in plant genome for the in-planta production of cry proteins (Bt proteins). Herbicide tolerance is achieved by introduction of the *CP4 EPSPS* genes into plant genome for the in-planta expression of CP4 EPSPS protein that enable the plant to survive in presence of herbicide (glyphosate). There are many ways to develop a GM crop, but the core steps remain same in all methods. **Figure 1.** contains the main steps involved in GM crops development.

Figure 1. Flowchart showing the main steps involved in GM crop development.

In mid-1990s after the commercialization of Bt corn, cultivation of transgenic crops dramatically increased, now GM technology have widely adopted by framers across the globe. Currently almost 14 million farmers are deliberately planting the GM crops in 25 different countries (James, 2009). 70% of cultivation land in china is used for the planting of GM cotton (Stone, 2008).

Insect resistant GM crops:





Insect resistance is mostly accomplished by the introducing the *Bacillus thuringiensis* (Bt) coding sequences in plant genome for the *in-planta* production of insecticidal *Cry* proteins (Bt proteins). These insecticidal crystal protein (ICP) is derived from a gram-positive, soil spore-forming bacterium of genus *Bacillus*. *Cry* protein target the various insect species like, Lepidoptera (butterflies, moths), Diptera (mosquitoes), Coleoptera (beetles), Hymenoptera (ants, wasps and bees,) (Schnepf et al., 1998) and nematodes (Wei et al., 2003).

Crystal protein is ingested by the target insect by consuming the Bt-plant tissues and in presence of Figure 2. Mode of action of the *Cry* Proteins specific receptor (brush-border membrane of epithelium cells), proteases, and alkaline condition in target insect’s gut create pores and paralyzed its digestive track that makes insect to starve to death (Dean, 1984). **Figure 2.** contains the overview of mode of action of the *Cry* protein. According to the integrated pest management (IPM) system, GM Bt-crops are environmental friendly and most effective bio-insecticides. It is also economically beneficial to famers due to reduced amount of pesticide (Kathage and Qaim, 2012).

Herbicide resistant GM crops:

Glyphosate (*N*-(phosphonomethyl)glycine) is an organophosphorus compound and used as broad-spectrum herbicide. Glyphosate entered in plants by foliage and interrupt the synthesis of essential aromatic amino acid (tyrosine, tryptophan and phenylalanine) by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme that results in death of the plants (Steinrücken and Amrhein, 1980). **Figure 3.**

An agrobacterium strain called CP4, produced a similar version of EPSPS enzyme (Pollegioni et al., 2011), which is resistant to glyphosate inhibition. Herbicide resistant GM crops are developed by the insertion of that *CP4 EPSPS* sequences.

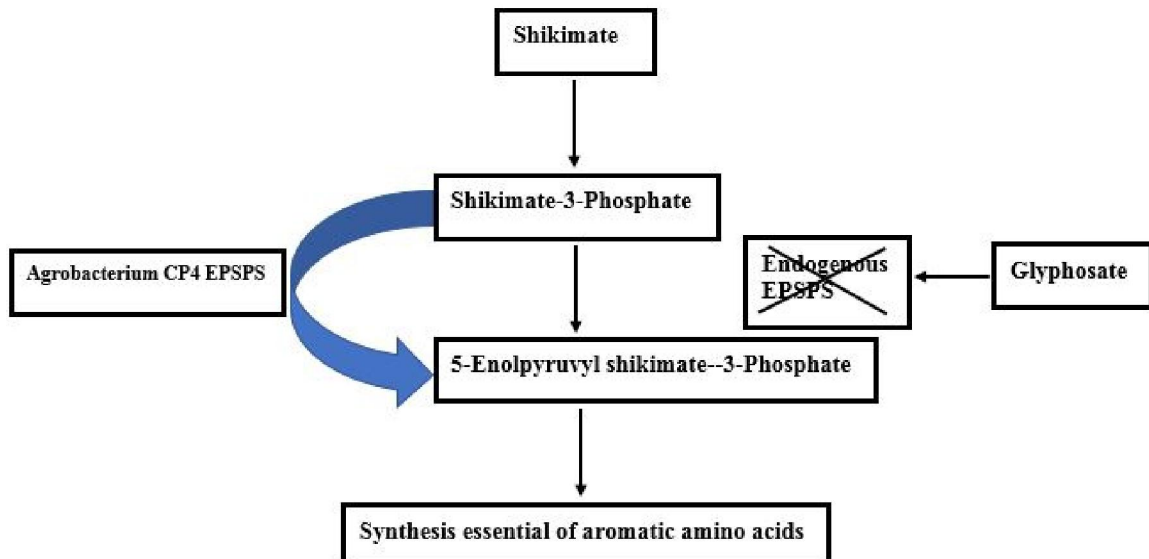


Figure 3. CP4 EPSPS enzyme’s mode of action

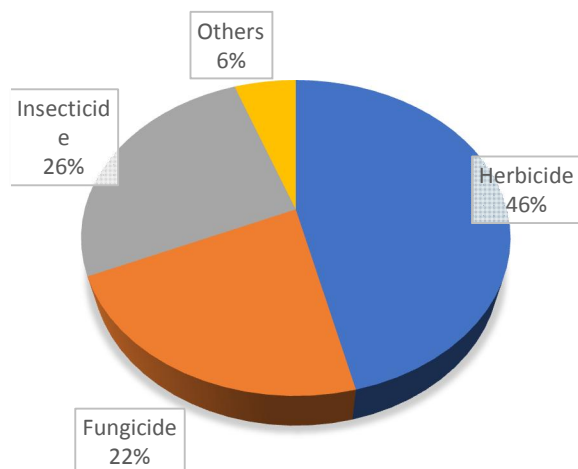


Figure 4

Figure 4. Pesticide market shares into plant genome for the in-planta expression of CP4 EPSPS enzyme. Weeds cause serious economic losses in crop production to farmers by reducing the crop yield due to competition with water, light and nutrients. Herbicides are one of the most effective method to control weeds and reduce the cost of farming (Gardner et al., 2006). The importance of herbicide can be judge by the losses in agriculture would be increased about 500% without the use of it. (Bridges, 1992, Bridges, 1994) In 2004, world-wide market of pesticides was recorded almost \$32.665 billion and about 45.4% of that market is accounted by the herbicide (Pacanoski, 2007).

Risks Associated With Gm Crops

Although the potential benefits of GM crops, promises they hold and its future application to satisfy the world's increasing population demands, these capabilities raises concerns about the potential hazards and unknown effect on ecology and human health

(Bennett et al., 2013). There is an opinion that the introduction of GMOs in the environment may cause survival and complication with adverse results (Arpaia et al., 2017, Hassan et al.). Ecosystems are complicated and environmental circumstances are random; these factors of ambiguity have caused some researchers and concerned public authorities to show concern about application of GMOs (Delaney, 2007). The risk assessment of GM crops has been majorly focused on

- Gene transfer from crops to wild plants and close species.
- Development of resistance in targeted subjects.
- Effect of transgenic plants on non-target ecosystems and organisms.

The main focus of the effect of transgenic plants was on soil microbes, soil community and soil ecosystem.

Risk assessment of the insect resistant (Bt) crops:

The adverse effect of Bt. protein on ecology and human health is negligible, many studies reported the rapid degradation of *Cry* proteins as it incorporated into soil and it is further supported by 40-year long history of safe use of Bt. microbial spray. Bt. proteins cannot affect the human and animal intestine because there are no such receptors and alkaline pH (animal and human intestine intend to have acidic pH) (Koch et al., 2015, Randhawa et al., 2011). Up to now extensive studies have been document on the risk assessment of Bt. crops related to non-target organism **Table 3.** Several articles were published to assess the potential effect of Bt. protein on mammals. Strict safety margins were drawn such as growth rate, weight gain, food intake, feed efficiency, blood chemistry, serum chemistry and histopathological analysis showed no lethal effect (Han et al., 2011, Liu et al., 2012, Song et al., 2015).

Table 3. Effect of Bt proteins on non-target organism (Yaqoob et al., 2016)

Experimental organism	Reference	Effect on Physiological Parameters			
		Development	Reproduction rate	Survival/ mortality	Body mass/size
Paddy grasshopper (<i>Oxyahyla</i>)	(Muhammad et al., 2010)	Harmless	Harmless	Harmless	Harmless
Honey bee (<i>Apis mellifera</i> L.)	(Han et al., 2010, Hof et al., 2008)	Harmless	Harmless	Harmless	Harmless
Ladybird (<i>Adalia bipunctata</i>)	(Schmidt et al., 2009)	Disturbance in larval development	Harmless	High mortality was observed in tested larvae	Reduced larval weight
Monarch butterfly (<i>Danaus plexippus</i> L.)	(Schmidt et al., 2009)	Harmless	Harmless	Harmless	Harmless
Aphid (<i>Aphidoidea</i>)	(Perry et al., 2010)	Reduced development	Harmless	Harmless	Reduced wing size/ body mass
Soil microbes	(Perry et al., 2010)	Harmless	Harmless	Harmless	Harmless
Earthworm (<i>Lumbricina</i>)	(Shen et al., 2006)	Harmless	Harmless	Harmless	Harmless
	(Zeilinger et al., 2010)	Harmless	Harmless	Harmless	Harmless

Risk assessment of the herbicide tolerance crops:

Many principle crops such as soybeans, maize, canola, alfalfa, and cotton were successfully transformed with *CP4 EPSPS* genes. Studies have shown both positive and negative impact but benefits outweigh the negative aspects so far. Glyphosate is superior to most of the herbicides and the least toxic that has it replaced (Henderson et al., 2010). Despite all these beneficial aspect, glyphosate resistant (GR) crops are responsible for the GR weeds known as superweeds. Almost 24 GR species are documented on six different continents. The *CP4 EPSPS* protein occur naturally in plants and found to be rapidly degradable when incubated invitro with digestive enzymes (Hammond et al., 1996b) and have no homology to any know allergen or toxin. The *CP4 EPSPS* enzymes is derived from the common soil bacterium *Agrobacterium tumefaciens*, so it's a not a novel exposure to soil. A common concern that introduction of the CP4 EPSPS into GM crops, might cause changes in it that can have an adverse effect human health and on environment. But phenotypically, compositional analysis and nutritional analysis suggest no unintentional effect on characteristics of GR plants (Assessment, 2010).

Extensive study have performed on risk assessment of the GR crops in association to mammals (Appenzeller et al., 2009, Hammond et al., 2004). Several physiological parameters such as growth rate, weight gain, food intake, haematology, serum chemistry, urine chemistry, morphology of organs and histopathological analysis were considered in most of the biosafety studies (Hammond et al., 2006). No adverse health effects were observed that ensured the safety of CP4 EPSPS enzyme (Appenzeller et al., 2008).

Conclusion And Future Prospect

Genetic engineering revolutionized the agriculture, by developing the GM crops to face numerous challenges such as increasing food demand, population growth, arable land and climate changes (Nicolia et al., 2014). The most common trail among GM crops is insect and herbicide tolerance that results in reduced use of insecticides and less management for weed control. Adoption of theses GM crops in agriculture raises global concerns about the environment and food security. In agriculture, the main aim of this technology is to develop GM crops which possess the desirable traits and have specific advantages over conventional crops such as, better nutritional profile, resistance to certain disease, pest, non-biological stress, longer shelf life and better yield (Magaña - Gómez and Calderón de la Barca, 2009). So, it is the prime priority to ensure the regulation and

biosafety of such biotechnological innovation (GM crops) before commercialization by the competent authorities worldwide like US Environmental Protection Authority (EPA), European Food Safety Authority (EFSA). (Arpaia et al., 2017). The primary biosafety assessment of the GM crops is ensured by the substantial equivalence analysis of it, in which GM ingredients were compared with their isogenic counterpart such as, compositional, molecular, phenotypical and agronomical trails. However, that analysis has limitation in safety assessment, because it cannot detect pleiotropic affect. So, animal testing in laboratories overcome this limitation and become important part in biosafety assessment of GM crops (Delaney, 2007). *Rattus Norvegicus* is the most extensively used model animal, to evaluate the any potential effect of GM crops on human health, because they share 95% genetic homology, similar enzymatic and cellular function like humans, and can mimic human disease. Scientific authorities (EFSA, 2008, Joint and Organization, 1996) recommended a 90-days feeding trail, that are used in many biosafety studies (He et al., 2009, He et al., 2008, Hammond et al., 2006). It is recognized that in biosafety assessment of the food additives, laboratory animal cannot be fed on whole food at the high level of exposure (Joint and Organization, 1996, Hammond et al., 1996a, Dybing et al., 2002). By feeding whole food to laboratory animal regardless of nutritional consequences, can results in unintentional and uninterpretable data that can compromise the true biosafety assessment (Pauli and Takeguchi, 1986).

In conclusion, risk assessment of the GM crops is inevitable. Advancement in molecular biology, nutrition, biochemistry, and toxicology hold the promise of providing new methodologies and tools (Magaña - Gómez and Calderón de la Barca, 2009). That will help in improvement and risk assessment of the GM crops without compromising the human, animal health and natural resources. Scientist needs to make more investigation and efforts to ensure the safety of these GM crops.

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