



Application of Genetic Engineering in Crop Improvement

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INTRODUCTION

Genetic engineering is also known as gene modification which was adding a new DNA to organism which was not already been exists in it, which can produce better quality yields that are resistant to biotic and abiotic stress through recombinant DNA technology. In 1973 first GMOs were bacteria generated and GM mice in 1974. In 1982 Insulin-producing bacteria were commercialized and genetically modified food has been sold since 1994. Genetic engineering techniques have been applied in numerous fields including research, agriculture, industrial biotechnology and medicine. Enzymes used in laundry detergent and medicines such as insulin and human growth hormone are now manufactured in GM cells, experimental GM cell lines and GM animals such as mice or zebra fish are being used for research purposes, and genetically modified crops have been commercialized (Blake et al., 2013)

To express specific traits genetic engineers have developed genetic recombination techniques to manipulate gene sequences in plants, animals and other organisms. Applications for genetic engineering are increasing as engineers and scientists work together to identify the locations and functions of specific genes in the DNA sequence of various organisms. Once each gene is classified, engineers develop ways to alter them to create organisms that provide benefits such as cows that produce larger volumes of meat, fuel- and plastics-generating bacteria, and pest-resistant crops (Acquaah, 2007)

According to (James, 2013 and Khan and Hakeem, 2015) Commercialization of first genetically engineered crop started back in 1996. Between 1996 and 2013 there has been more than 100 fold increase in the acreage of genetically engineered crops.

The first genetically modified crop plant was produced in 1982, an antibiotic-resistant tobacco plant. The first field trials of genetically engineered plants occurred in France and the US in 1986, tobacco plants were engineered to be resistant to herbicides. One of the most vivid examples of domestication is maize (corn). Beginning some 6,000–10,000 years ago, ancient Meso-American farmers drastically changed teosinte (*Zea mays* subsp. *parviglumis*) through selection. Teosinte is a grass species that has numerous lateral branches and cobs with 5–12 individually encapsulated kernels that drop to the ground when ripe. Through human selections based on very rare, desirable attributes caused by naturally occurring mutations, a plant was derived with no lateral branching (that is, a single stalk) and a cob with dozens or even hundreds of large seeds (kernels) that were encased in husk leaves; this resulted in the maize that is grown today (Doebley, 2004; Flint-Garcia, 2013; Wang et al., 2015).

THE DEVELOPMENT OF GENETIC ENGINEERING IN AGRICULTURE

People have been domesticating plants for at least 10,000 years. Early plant domestication involved selecting individual plants, fruits, seeds, inflorescences, or other propagules for characteristics of interest. Selected characteristics (traits) included higher yields, reduced toxicity, improved flavor or morphology of seeds or fruits, and seed heads (in grains) or pods (in legumes) that did not shatter and were therefore easier to harvest. Selection permitted people to domesticate numerous wild plants into crops, such as wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), potato (*Solanum tuberosum*), and tomato (*Solanum lycopersicum*).

Genetically Engineered "insect resistant" Rice

The introduction of Bt genes into rice is discussed as a solution to the problems with *S. incertulans* (ESTRUCH et al. 1997; NAYAK

et al. 1997) and is seen as a contribution to integrated pest management. The often used term "insect resistant" plants is misleading because genetically engineered plants with Bt genes are produced to be toxic for one or a few insect species. Plant pathogenic insects from groups like grasshoppers, aphids, and some beetles are not susceptible to the Bt toxins. While herbicide resistance works via the resistance of the plant towards a new herbicide and therefore fixes the use of chemical production factors "insect resistant" plants produce their own insecticides. Manufacturers claim that the cropping of Bt plants will lead to a significant reduction or even elimination of synthetic insecticides in intensive agriculture. If this will be true with such highly selective plants is questionable. In intensive agricultural systems, insecticides will still be used in order to control pathogenic insects which are not susceptible to Bt. Furthermore it has to be clear that Bt plants are only of value to farmers as long as no cheaper means of insect control are on the market. The main reason for planting Bt crops is an anticipated cost advantage and not the reduction of environmental impacts.

Rice genetically engineered to resist heat waves can also produce up to 20% more grain

When plants are exposed to light, a complex of proteins called photosystem II (PSII) energizes electrons that then help power photosynthesis. But heat or intense light can lead to damage in a key subunit, known as D1, halting PSII's work until the plant makes and inserts a new one into the complex. Plants that make extra D1 should help speed those repairs. Photosynthesis that takes place in chloroplast have their own DNA, including a gene for D1, and most biologists assumed the protein had to be made there. But chloroplast genome is much harder than the plant cell nucleus.

A team led by the plant molecular biologist Fang-Qing Guo of the Chinese Academy of Sciences bet that D1 made by a nuclear gene

could work just as well—and be made more efficiently, its synthesis takes place in cytoplasm rather than the chloroplast. Guo and colleagues tested the idea in the mustard *Arabidopsis thaliana*. They took the D1 gene from chloroplast and insert into a DNA stretch away from nucleus which was heat stress tolerant.

They had found that modified *Arabidopsis* seedlings could also survive extreme heat in the lab—upto 8.5 hours at 41°C—which killed most of the control plants. Same gene *Arabidopsis* also protected in the tobacco and rice. In all three species, photosynthesis and growth decreased less than in the surviving control plants. In 2017 temperature exceeds 36°C in Shanghai transgenic rice planted in test plots yielded 8% to 10% more grain than the normal control plants.

A shock that happened at normal temperatures was that engineered plants of all three species had more photosynthesis—tobacco's rate increased by 48%—and grew more than control plants. The transgenic rice yielded up to 20% more grain in the field. "It truly surprised us," Guo says that. "He felt that they have caught a big fish"

Genetically modified cotton: How has it changed India?

Nearly twenty years ago, Bt cotton was introduced in India. Genetically modified cotton which produces more yield. Strains of the bacterium *Bacillus thuringiensis* produce toxins that are harmful for a variety of insects. Combat bollworm- larva that will attack the cotton plant. To reduce the insecticide needed in cotton a new GM crop has been introduced.

Most of the crops grown in India are with Bt technology. Nearly 80% of the crops growing in India were with Bt. Technology.

The effects of Bt cotton

From the past 15 years effect of Bt cotton were studying. There is broad consensus among scientists that the introduction of Bt cotton has had a positive effect on cotton production, trade, farmers' livelihoods, and the

environment. One of the study says that the Bt cotton has using less insecticide upto 37%, increased crop yields by 22%, and also increasing the farmers yield by the 68%. Some studies says that the Bt cotton that don't enduring the income to Indian farmers. Although it reduces the insecticide usage initially and later they have gained insect resistance. There has been a notable gaps between the crop production in central and state level.

Novel approach to studying Bt cotton effects

To bridge these gaps, Professor Ian Plewis of the University of Manchester investigated the effects of Bt cotton on farmers across different regions in India. Drawing on information from the Cost of Cultivation Surveys, the Cotton Advisory Board, and the Directorate of Economics and Statistics, he collected information about insecticide use, yield, and profit. Prof Plewis also drew on several sources to obtain information about Bt cotton adoption in each state. This research mainly focus on the three main aspects of farming outcomes –investment on insecticides, yield, and also profit—mainly in Punjab, Haryana, Rajasthan in north India. 16% of the cotton takes place in this state's.

Advancing previous research, this work used annual time series methods of cotton statistics over several years, rather than comparing statistics at two time points which ignores year-to-year changes. In addition, using advanced statistical methods, Prof Plewis explored the effects of Bt cotton at the state level, rather than the national level, allowing for more detailed regional information

Prof Plewis crucially stated that the picture of Bt cotton effects across India varies state-by-state. The research suggests that the effects of Bt cotton have not been entirely beneficial to farmers' yield and profits in all states, as has previously been assumed. In contrast, across most states Bt cotton achieved its primary purpose to reduce insecticide use by farmers. Therefore, assertions by anti-GM (genetic modification) groups and previous.

Genetic Engineering Application in Potato Breeding

Increasing resistance to viruses (by coat protein gene)

These are the major limiting factors in quality and yield in susceptible potato cultivars. Several new ideas have been developed that increase the yield and other characters in solanum. First release of GM potatoes in the USA. First approach was the transformation of the viral coat genes which was derived of pathogen resistance and act like as a cross protection (Pierpoint, 1996). Transformation with the coat protein gene of Potato virus X (PVX) was one of the first attempts to obtain pathogen-derived resistance to a major potato virus (Hermenway et al., 1998). First genetically modified potatoes were virus-resistant potatoes in Netherlands. Cultivation tests have to done for the Transgenic potatoes with resistance to PVX and will be ready for commercial introduction within a few years, as reported by Bijman (2000). Resistance to potato leafroll virus (PLRV) was also investigated after insertion of the coat protein gene of PLRV into the genome of potato. In tested plants a detectable level of coat protein was not accumulated, virus-infected transgenic plants contained markedly lower levels of viral antigen than control plants; this resulted from a reduced rate of virus multiplication in the transgenic plants (van der Wilk et al., 1991). For the introduction of resistance to Potato virus Y (PVY), coat protein gene-induced resistance was applied in several cases (Beachy, 1997; Józsa et al., 2002). Under normal conditions potato plants were infected frequently. Although a detectable level of coat protein was not accumulated in any of the tested plants, PVX and PVY infection in potato may result in severe loss in the certification of seed potatoes and affect quality and yield in commercial production. Lawson et al. (1990) transformed a major commercial cultivar, Russet Burbank, with the coat protein genes of PVX and PVY. After mechanical

inoculation both the viruses were resistant where the transgenic plants will have the CP genes. One line was also resistant when PVY was inoculated with viruliferous green peach aphids.

Increasing resistance to fungi and bacteria

late blight caused by *Phytophthora infestans* was the serious disease in potato. when conditions are warm and moist it can spread extremely rapidly, leading to devastating losses. New, adapted forms were formed when owing to its flexibility, the disease has been able to survive every management strategy used thus far and has responded with new. Today, the disease is combated using fungicides and heavy metal treatments. Later genetic engineers have come up with a promising new strategy.

By generating transgenic plants we can control fungal infections which are carrying gene *ac2* from amaranth, *Amaranthus caudatus* (Lipkova et al., 2001). The expression of this gene results in a protein which is highly homologous to the cysteine/glycine-rich domains in the chitin-binding proteins (Broekaert et al., 1992). The binding of these to the chitin localized in internal fungal cell walls caused an alteration in their polarity and finally inhibited the growth of the fungi (Selitrennikoff, 2001).

chitinases are one of the major classes of pathogenesis-related (PR) proteins in plants, which play important roles in plant defence against infection by pathogens (Melchers et al., 1994; Neuhaus, 1999). Chye et al. (2005) reported the evaluation of potato lines transformed with the *Brassica juncea* chitinase and *Hevea brasiliensis* beta-1,3-glucanase genes. They demonstrated that young transgenic potato plants co-expressing either or both of the genes showed healthier root development than untransformed plants in soil infected with *Rhizoctonia solani*.

Wet rot of bacterial origin and potato blight which was enhanced by the antimicrobial peptide called Temporin A. This is a disease caused by the fungus *Phytophthora*

erythroseptica and the bacterium *Erwinia carotovora*. Results that have been stated transgenic potato plants that express temporin A can serve as a good tool for the control of the most significant fungal pathogens such as *P. infestans* and *P. erythroseptica* (Osusky et al., 2004).

Defending plants against pathogens by over-production of hydrogen peroxide in plants is another approach. In the presence of molecular oxygen, glucose oxidase catalyses β -D-glucose oxidation, releasing gluconic acid and hydrogen peroxide. The glucose oxidase gene from *Aspergillus niger* was tested from this aspect. Potato plants that produce hydrogen peroxide were characterized by increased resistance to potato blight (*P. infestans*) and to bacterial rot caused by *Erwinia carotovora* (Wu et al., 1995).

Increasing resistance to pests

Potato beetle (*Leptinotarsa decemlineata*) is one of the most consequential potato plant pests, which often becomes resistant to chemical. Cry3A gene in GM potatoes which was originating from the soil bacterium *Bacillus thuringiensis* (Bt), were useful to control this beetle. In the leavestoxic protein will be present; after ingestion by the potato beetle, it passes into the intestines and causes the death of the pest. This protein affects all the developmental stages of potato beetles in the same way, but does not affect their natural enemies (Perlak et al., 1993). Several GM potato cultivars with improved resistance to the potato beetle have been approved in the US and in Canada (Côté et al., 2005; Romeis et al., 2008). Potatoes carrying genes for the production of other insecticide proteins have also been developed, such as snowdrop (*Galanthus nivalis*) lectins (GNA), wheat α -amylase inhibitors (WAI) and bean chitinases (BCH). The insecticidal capability of these transgenic plants was tested in peach-potato aphid (*Myzus persicum*). The best insecticidal effect was recorded for genes that code the lectins (GNA) from snowdrop. In a subsequent study the influence of transgenic potato plants

expressing the above-mentioned proteins in the larvae of the moth *Lacanobia olearacea* was tested. All the plants expressing GNA showed an enhanced level of resistance. These results support the hypothesis that GNA has a significant adverse effect on insects (Gatehouse et al., 1996; 1997).

The potato tuber moth, PTM, *Phthorimaea operculella* (Zeller), is one of the most damaging potato pests in tropical and subtropical areas, while *Symmetrischema tangolias* (Gyen), another PTM species, is a serious potato pest in the Andean region. On potato foliage, stems and tubers damage can be seen. Effective Bt strategy has proved in reducing PTM infestations in stores. The expression of the Bt genes confers non-conventional host plant resistance to this pest. Lagnaoui et al. (2000) evaluated the effect of Bt-cryIIa1 (cryV, now designated cryIIa1 under the revised nomenclature) transgenic potato plants on the two species of potato tuber moth mentioned above. Detached leaf bioassays were done using 10 neonate larvae per replication on each transgenic line of the potato varieties Atlantic and Spunta. The mortality in Atlantic transgenic plants was lower for *P. operculella* (ranging from 18 to 34%), than for *S. tangolias* (ranging from 40 to 94%). High level of mortality in all transformed Spunta lines in both species having mortality range of 80 and 98%. The results of both PTM bioassays demonstrated that high levels of Bt-cryIIa1 expression can be achieved with the gene construct and vectors used in Spunta transgenic lines. Durable resistance to PTM and other insect pest was offered by the Bt-cryIIa1 gene.

Increasing resistance to herbicides

Reduction of pesticide use was the major challenge to farmer. Besides genotypes resistant to insect pests and microbial pathogens, lines resistant to herbicides have also been generated. The validity of such modifications consists above all in the potential application of a herbicide in the most suitable period with the concurrent maximum

reduction in weeds (Slater et al., 2003). The insertion of the bar gene (PAT) from the bacterium *Streptomyces hygroscopicus* into

potato plants is an example. Modified potato plants proved to be resistant to the herbicide phosphinothricin (Padegimas et al., 1994).

SOME OF THE CROPS THAT ARE GENETICALLY MODIFIED

Genetically Conferred Trait	Example Organism	Genetic Change
APPROVED COMMERCIAL PRODUCTS		
Herbicide tolerance	Soybean	Glyphosate herbicide (Roundup) tolerance conferred by expression of a glyphosate-tolerant form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) isolated from the soil bacterium <i>Agrobacterium tumefaciens</i> , strain CP4
Insect resistance	Corn	Resistance to insect pests, specifically the European corn borer, through expression of the insecticidal protein Cry1Ab from <i>Bacillus thuringiensis</i>
Altered fatty acid composition	Canola	High laurate levels achieved by inserting the gene for ACP thioesterase from the California bay tree <i>Umbellularia californica</i>
Virus resistance	Plum	Resistance to plum pox virus conferred by insertion of a coat protein (CP) gene from the virus
PRODUCTS STILL IN DEVELOPMENT		
Vitamin enrichment	Rice	Three genes for the manufacture of beta-carotene, a precursor to vitamin A, in the endosperm of the rice prevent its removal (from husks) during milling
Vaccines	Tobacco	Hepatitis B virus surface antigen (HBsAg) produced in transgenic tobacco induces immune response when injected into mice
Oral vaccines	Maize	Fusion protein (F) from Newcastle disease virus (NDV) expressed in corn seeds induces an immune response when fed to chickens
Faster maturation	Coho salmon	A type 1 growth hormone gene injected into fertilized fish eggs results in 6.2% retention of the vector at one year of age, as well as significantly increased growth rates

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