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Applications of Genetic Engineering and Biotechnology:

Genetically Modified (GM) Cotton

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1. Preface

1.1 Our Motivation

When our teacher Dr. Patrick Ruggle first introduced us into the topic of biotechnology, he brought a “GM tomato”, asking whether anyone wanted a slice. We were both afraid of the impacts it might have had on our health since genetic modification indeed seemed to be harmful to us. Moreover, we were wondering what other GM crops would look like, especially those which are not edible, such as cotton, as these appear less harmful than food crops. We wanted to figure out what influence GM cotton has on human beings and what impacts it has on humans when it is worn.

1.2 Interesting Facts

With the use of GM crops, such as Bt cotton, there was no need anymore in using pesticides, which were previously known to be health hazardous. Hence, GM crops have manifested to be a more environmentally-friendly alternative. However, ever since Bt cultivation has been an approved technique in India, the suicide rate of farmers in India has increased to a drastic level. The Indian Biotech opponents have attributed the increase of suicide to the monopolization of GM seeds. While recombinant DNA technology seems to be beneficial for everyone at first glance, it appears to permit seed producing companies to become monopolies, such as Monsanto in India, which made farmers obliged to buy these cotton seeds, causing farmer indebtedness, as well as privatization of seeds. The top three companies (Monsanto, DuPont, and Syngenta) represent 53% of the seed market. In 2009, Monsanto sold 90% of their GM seeds worldwide. These recent actions have opened the debate on whether we should continue with Bt cultivation or not. Are they really as beneficial as advertised? Besides that, while pesticides are considered as hazardous, one might question whether GM crops have any impact on health as well.

1.3 Key Questions

- What are the applied techniques to produce GM cotton?
- Is Bt cotton more beneficial than detrimental?
- Will the distribution of benefits be fair, or will some persons benefit at the mercy of others?

2. Introduction

2.1 Demand

Cotton is the most profitable non-food crop and is the essential raw material which produces the primary fibre responsible for clothing throughout the world. Furthermore, the cotton fibre, as well as the cotton seeds, have their use ranging from cosmetics, electrical equipment to livestock feed. Therefore, ever since the demand in the fibre market increased (See Fig. 1), due to the growing population and consumerism, the cotton plant itself is redesigned to keep place.

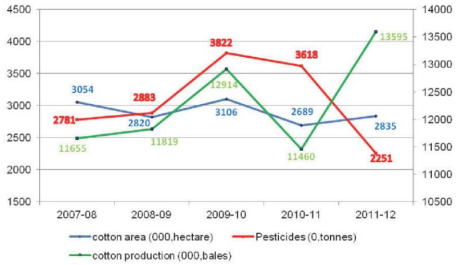


Figure 1: The trend in the relationship between cotton acreage, consumption of pesticides, and production in bales.

Figure 2: A depiction of the pink bollworm Pectinophora gossypiella.

Yet, farmers had already been losing their cotton, due to pests, such as tobacco budworms *Helicoverpa armigera*, cotton bollworms *Helicoverpa armigera*, and pink bollworms *Pectinophora gossypiella* (See Fig. 2). Now, that the demand has increased, any bad harvests or crop failures could be a significant threat, since the carrying capacity is not progressing, along with the growing population. Thus, new strategies had to be invented to cultivate the crops more efficiently. These have been accomplished through the following achievements throughout centuries.

2.2 Scientific History

It was Shigetane Ishiwatari, a Japanese biologist, who successfully isolated *Bacillus thuringiensis* (Bt) as the cause of the sotto (sudden-collapse) disease in 1901. Nevertheless, in 1911, Ernst Berliner was the one who gave the name to the bacterium when he managed to isolate a bacterium that had killed a Mediterranean flour moth and rediscovered Bt. *Bacillus thuringiensis* (Bt) was named after the German town Thuringia where the moth was found. Four years later, Berliner reported that crystal could be found within Bt, but the activity of it remained unknown until then. In 1920, Farmers found the use of Bt as a pesticide. During the ‘80s scientist became aware that insecticides and pesticides chemicals were harming the environment. Furthermore, studies have shown that insects became increasingly resistant to synthetic insecticides. Thus, the U.S. government commenced funding researches on Bt. Since the advancement in molecular biology in 1995, scientists soon were able to insert the gene that encodes the toxic crystals into a plant.

In the same year, the U.S. encountered a loss of cotton by over 4% which is above a quarter billion dollars’ worth of cotton. As a result, to meet the standards, the U.S. agrochemical multinational Monsanto developed Bt cotton, one of the first genetically engineered crops in 1996. The idea was to create cotton which is resistant to the common pests, in order to reduce the losses of cotton worldwide. In the same year, U.S. agriculture was introduced to Bt cotton.

Nowadays, not only American farmers switched from conventional cotton production to Bt cotton, but also cotton growers from India, China, Brazil, and Africa are among the 16 countries with commercial plantation of Bt cotton. Nevertheless, Bt technology is not being permitted in some countries in Europe, as there are restrictions on its use. Many field trials have reported that Bt cotton yield is 6-25% higher than traditional cotton varieties. In addition, the Bt cotton obtained is more than non-Bt cotton, because the genetically modified cotton minimizes the pest damage by increasing the resistance of the plant.

2.3 Alternative Treatments

Conventional and organic cotton are the two-alternative crop to Bt cotton. The production of regular cotton takes 24% of the world’s insecticides and 11% of the world’s pesticides. However, the majority of these go into the air, soil, and water around the fields and lead to the ground being contaminated, as well as harming and killing animals and farmers exposed to these contaminants.

The difference between conventionally and organically grown cotton is that pesticides and insecticides are used in conventional cotton, whereas in organic cotton, they are not. Besides that, since there is no protection, the production of organic cotton requires a significant amount of preservation and care, as well as take the most risk, which is why their prices ought to be higher.

3. Description of Engineering Technique

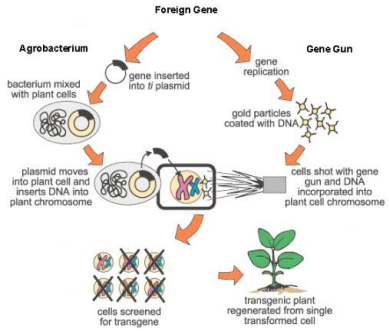


Figure 3: A depiction of applied engineering techniques.

There are four ways of transferring genes in cotton crop plants, such as plasmid method, particle bombardment, direct DNA uptake, and microinjection. The particle bombardment method is achieved by delivering foreign DNA into plant cells through high-velocity metal particles, while the Agrobacterium-mediated process of DNA transfer provides the transgenes in plasmids, including the isolated genes of interest which were obtained from DNA libraries and amplified using PCR (polymerase chain reaction) methods. (See Fig. 3) In this method, a chemical (antibiotics) has to be inserted to ensure that only the cells which have taken up the transgenes will survive. The other two methods (direct DNA transfer and microinjection) are less commonly used.

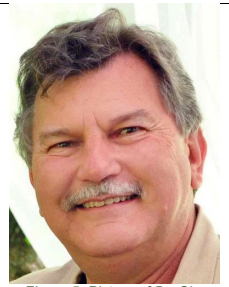
The principle of the bacterium *Bacillus thuringiensis* (Bt) from a biological perspective is as follows. Now that the plant's genome is genetically modified, it developed the ability to express insecticidal proteins from strains of the bacterium *Bacillus thuringiensis*, which include crystal (Cry) toxins, cytolytic (Cyt) toxins, and other vegetatively expressed insecticidal proteins (vip), depending on specific insect species. A total number of 342 Bt toxin genes have been developed to protect the GM crops from insects. For cotton plants, this is done by transferring the Bt gene Cry1Ac into the genome of cotton explants using the bacterium *Agrobacterium tumefasciens.* While Cry1Ac toxins affect specific insects at the species level, they are known not to harm any non-target species, such as human beings. The major pests are the three bollworms, American bollworm *Helicoverpa armigera*, pink bollworm *Pectinophora gossypiella,* and the spotted bollworms, *Earias vittellaand* and *Earias insulana,* which were known to be severe threats to cotton production and thus significant for yield losses. Bt cotton hybrids also exist which are derived from technologies developed by Monsanto (Cry1Ac and Cry1Ac + Cry2Ab), Metahelix (Cry1C), JK seeds (Cry1Ac) and more. A total number of 1128 Bt cotton hybrids existed in 2012.

These substances dissolve the gut lining of insects when ingested. The high pH environment of the insect's gastrointestinal system activates the toxins which bind to specific cadherin receptors located on the brush border membrane of the insect's midgut. As a result, ion channels are formed, allowing potassium ions to leak through the pores in the epithelial membrane. With the loss of crucial potassium ions, required for cell respiration, the affected cells lyse and die, enabling bacteria and Bt spores to enter the insect's body cavity and cause death by internal infection. However, Cry toxins are only specifically toxic to particular classes of insects. For instance, the Cry1Ac appears to be toxic to the three cotton bollworms, but less poisonous to the tobacco caterpillar *Spodoptera litura.* On the other hand, different Cry toxins such as Cry1F, and Cry1C are more lethal to the tobacco caterpillar *Spodoptera litura* and relatively less toxic to the cotton bollworms. Accordingly, Bt cotton is only selectively toxic to insects, which is why hybrids are created in order to increase toxicity as much as possible.

4. Documentation and Pictures of Research Institutions Contacted

After several inquiries to different institutions and experts, three experts agreed to answer the ten questions that we prepared for the interviews.

4.1 Interview Partners

Dr. Monika Messmer	Dr. Ciro Antonio Rosolem	Mariana Gomes
 *Figure 4: Picture of Dr. Monika Messmer*	 *Figure 5: Picture of Dr. Ciro Antonio Rosolem*	 *Figure 6: Picture of Mariana Gomes*

• Frick, Switzerland
• Research Institute of Organic Agriculture FiBL
• Department of Crop Science
• Activity Areas:
  ○ Group lead Plant Breeding
  ○ Plant breeding for organic agriculture
  ○ Cultivation of medicinal plants
	• São Paulo, Brazil
	• Agronomist, agricultural studies educator
	• Works at the Department of Crop Science, São Paulo State University
	• Activity areas:
	  ○ Agronomy, Applied Crop Physiology, Plant Nutrition and Fertilization, and Soil Science
	  ○ His current project is "Enhancing Phosphorus, Nitrogen and Potassium Use Efficiency in Agricultural Systems
		• São Paulo, Brazil
		• Student at São Paulo State University
		• Currently working on her master's degree in Genetics and Plant Breeding
		• Activity areas:
		  ○ Student in Agricultural Microbiology

Table 1: Information Chart of the Interview Partners

4.2 Interviews

The following interviews were all conducted by email. Their original answers are found below.

1) What are the engineering techniques?

- *Dr. Monika Messmer:* "At the moment there are genetic engineering with *Agrobacterium tumefaciens* transfer, main traits are stacked Bt genes against bollworm and herbizide resistance. New methods like CRISPR CAs are in the experimental stage."

- *Dr. Ciro Antonio Rosolem:* "There are several techniques. The main ones are cell bombing and use of microorganism to transfer the genes."
- *Mariana Gomes:* "Some kind of bacteria is used to implant special genes into crops. But there are also other techniques."

2) How could one make sure that interbreeding between organic and GM cotton occurs?

- *Dr. Monika Messmer:* "We try to avoid outcrossing of GM into organic cotton by thorough GM testing of seeds (qPCR before sowing), ELISA or strip tests in the field during cultivation, harvest, samples before ginning, qPCR before and after ginning.
  We try to isolate seed production as much as possible to avoid cross pollination. This is done by geographic distance from GM cultivation and plant barriers like borders of sugarcane, maize etc.
  We also try to breed diploid cotton species that do not intermate with tetraploid GM cotton."
- *Dr. Ciro Antonio Rosolem:* "Organic cotton and GM cotton are completely different things. Organic cotton is grown without synthetic fertilizers and pesticides. GM cotton is a cotton that had a gene from other species introduced. Usually organic people do not grow GM crops. Therefore that is no point interbreeding organic and GM cotton."
- *Mariana Gomes:* "I do not think people would want to achieve that. Because this would make sure that everyone has access to these seeds. Plus GM crops are far separated from organic crops."

3) Would you be able to tell GM cotton apart from organic cotton?

- *Dr. Monika Messmer:* "Yes, if he makes a strip or qPCR test."
- *Dr. Ciro Antonio Rosolem:* "Absolutely. From organic or conventionally grown cotton. Rather more difficult would be to tell apart conventional from organic cotton."
- *Mariana Gomes:* "From appearance, no. From genetic perspective, yes."

4) Does GM cotton affect biodiversity if they are cultivated without restrictions?

- *Dr. Monika Messmer:* "Yes, the events are patented and not freely available. Also breeding companies introgress the event in only few lines with are extensively used for hybrid seed production. The whole research concentrated on only one species the tetraploid *G. hirsutum*. Severe consolidation of multinational companies displaces with their GM cultivars local or public varieties in different continents."
- *Dr. Ciro Antonio Rosolem:* "There is no danger. Biodiversity can be temporarily affected in the surroundings of the GM crop. Biodiversity is less affected by GM crops than by pesticide applications. However, it is recommended to crop at least 20% of the area with non-GM, to preserve biodiversity."
- *Mariana Gomes:* "I think it even increased biodiversity. The pesticides are harmful for environment. So with use of GM crop, surrounding plants are preserved."

5) Does gene modification have any health impacts (i.e. causing diseases)?

- *Dr. Monika Messmer:* "To my knowledge has Bt no health effect. But herbizide resistance increased the application of herbizides which have a negative impact on health, see resent law suit against glyphosate of Monsanto."
- *Dr. Ciro Antonio Rosolem:* "Absolutely not."
- *Mariana Gomes:* "No."

6) What are the advantages/disadvantages of GM cotton?

- *Dr. Monika Messmer:* "Advantage: resistance against bollworm, at least for several years. In India there are meanwhile an outbreak of resistant bollworms
  Disadvantage: patent, makes farmer dependent to buy seed each year. Fosters monopolization of seed market as development and registration is very cost intensive. Bayer/Monsanto offer seed, fertilizer and pestizide in one package, their sales man offer credits with high interest rates to farmers, this makes farmers very dependent and vulnerable if the harvest is lower than expected (indebtedness). Meanwhile sucking pest are much larger problem than bollworm. Large scale application of Bt gene leads to resistance of bollworms. Public breeding had almost stopped."

- *Dr. Ciro Antonio Rosolem:* "Pest control is very specific, not affecting the natural enemies of the pests. Usually, it is less expensive and easier to manage. It has been estimated an increase of 377 million tons of grains and fibers in the last 17 years due to the introduction of GM crops (not only cotton). Around 123 million ha were saved, i.e., forests were not put down, due to the use of GM crops, and 497 million tons of pesticides were not applied to agricultural lands. Furthermore it was avoided the emission of 26.7 million tons of CO_2 (Green-House gas) into the atmosphere."
- *Mariana Gomes:* "Advantages like better yields and better cotton production thereby and no insects attack that could create bad harvest.
  Disadvantages like every GM crop uses different modified genes. This is why pesticides have special levels of toxins affecting special insects. Meaning not every insect is killed by the GM crop."

7) Does GM cotton have a future (despite of the negative side effects if there are any)?

- *Dr. Monika Messmer:* "I guess it has a future as especially in the US herbizide resistance reduces labor cost and eases machine harvest. However, public is more and more afraid of side effect of pesticide in the air, water and soil."
- *Dr. Ciro Antonio Rosolem:* "There are very few side effects, mainly in agricultural lands, and so, yes GM cotton has a present and a future."
- *Mariana Gomes:* "Definitely yes. There are only a few side effects that can occur to normal cotton too like droughts."

8) Which country has the most potential in becoming the most successful GM cotton producer?

- *Dr. Monika Messmer:* "USA, India have more than 95% GMO production. "
- *Dr. Ciro Antonio Rosolem:* "Most of the cotton producing cotton such as U.S., China, India, Pakistan, Brazil, Australia, Uzbekistan, etc."
- *Mariana Gomes:* "India, China, U.S and Brasil of course."

9) Are there any alternative treatments to GM cotton?

- *Dr. Monika Messmer:* "In countries without GM cotton, conventional farmers apply pestizides. But they also need to spray insectizides as Bt is not working against sucking pest, and even sometimes against resistant bollworms."
- *Dr. Ciro Antonio Rosolem:* "The alternative is the use of pesticides, that are less specific, and can kill the pest's natural enemies. Or the use of more herbicides, increasing costs and environmental risk."
- *Mariana Gomes:* "Yes, pesticides. But again, it can poison other organism (also humans) because of application. So I would stay with GM cotton."

10) What would you prefer to buy: GM or organic cotton?

- *Dr. Monika Messmer:* "This depends on the market. Presently consumers look for organic cotton, but also for cheap cotton. Organic production is more labor and knowledge intensive and has higher risk of crop losses. Therefore price of organic cotton need to be higher."
- *Dr. Ciro Antonio Rosolem:* "There is no point in making this kind of choice. The question should be: Conventional or organic cotton? Conventional cotton can be GM of not.
  So, there can be a choice to by conventional non-GM cotton or conventional GM cotton. Or to buy organic or conventional cotton. They are all the same. There is no difference in the cotton you buy."
- *Mariana Gomes:* "I do not think it makes much of a difference. You cannot tell GM cotton from organic cotton apart in the market, so this should not be a bother really."

5. Discussion

5.1 Progress Made with the Application of Engineering Technique

Bt cotton has only been used for more than 20 years, and so far, it allowed an expansion in yield of 6% to 25%, making GM crops the fastest adopted crop technology worldwide.

Even the latest traits, along with pesticide resistance, such as drought-tolerance and non-browning are being developed and have been commercially approved, which may permit further improvements and economic profits.

5.2 Future Research Steps

While the transformation method has successfully worked for many Bt cotton seeds, researchers seek for even better, less time-consuming methods, since it takes between 8 and 12 months to generate the desired genomes.

A newer, more experimental method is the targeted genome editing for cotton improvement using targeted mutagenesis through CRISPR/Cas9 systems. Since cotton is a complex tetraploid, it is tough to duplicate its genome, initiating, the application of CRISPR/Cas9 in cotton a rather technically challenging genetic transformation process. But the intention is to efficiently create point mutations which directly lead to the desired traits for Bt cotton.

Overall, more studies on the impacts of Bt cotton needs to be done in order to guarantee its safety. It has only been approximately 20 years of research. It is not clear whether toxin resistance of certain insects will persist in the next 30, 40, or 50 years or not. Subsequently, it has shown that evolved resistance tends to have been increasing recently.

5.3 Ethical Aspects

Nevertheless, Bt cotton has advantages, as well as disadvantages for each party. For instance, producers do not have to use pesticides anymore, which reduces costs. Moreover, they possess the freedom to adopt. Yet, a reduction of the price may arise, due to abundant supply. Not to mention, there is no way to save seeds. For consumers, the use of Bt cotton means reduced environmental pollution from the decreased application of pesticides, resulting in improved environmental quality. Nonetheless, the patented seeds may demand higher prices in the market.

Not only does Bt cotton influence us, but it also affects the environment, such as increased sustainability and maintenance of biodiversity. In addition to that, reduced air and water pollution are significant changes which provide a more environmentally-friendly future. Despite that, there is an extended threat with respect to gene flow quality, resulting in a population change.

5.3.1 Advantages

- Replaces the use of synthetic pesticide in the environment, which is a more environmentally-friendly approach
- Toxin expression is contained within the plant system. Hence, only insects feeding on that crop die
- Bt cotton has a higher resistance to pests, due to the toxins
- GM cotton requires fewer sprays of chemical pesticide than for standard variety; time-saving
- Lower production costs (pesticides are expensive!) and thus reduces energy use
- Global adoption of Bt cotton expanded drastically
- Increase in cotton production

5.3.2 Disadvantages

- Monopoly in the market (only a few enterprises producing Bt cotton seeds)
- Farmers are highly dependent on the companies, aggravating poverty
- Advertisement on insecticide manufacturing companies
- Rising price costs while yield levels maintain low, due to insufficient water resources
- Unemployment, farmer indebtedness, let alone, farmer suicide
- Ineffective against certain species, such as sucking pests like aphids or whitefly, etc.
- Promotes malpractices
- Risk of gene flow
- Throughout time, some insects have grown resistance against these toxins

6. Summary

When the first scientist developed the new trend of genetic modification, this new discovery opened a new field in agricultural and crop science. Thanks to genetically modified crops, also known as transgenic crops, the number of crucial resources increased to a significant level to fulfil the needs of the growing population. Not only that, but the new seeds have also boosted the economy in terms of efficiency and production, significantly by reducing the chance of bad harvests and crop failures. Besides this, some consumers are still sceptical about high-tech crops.

In this respect, alternative treatments also exist, such as the use of conventionally or organically grown cotton instead of genetically modified cotton. While these two options are much more prone to pest and insects, they are much more challenging to cultivate. Especially the organic ones require maintenance to a large extent. Despite the restrictions in the application of Bt technology in some European countries, Bt crops have been a success worldwide. But how are the crops genetically modified in the first place? What techniques are being used?

The process of gene modification is as follows. The gene of interest which carries the desired traits, such as bollworm resistance in Bt cotton, are identified and isolated. The DNA information is then copied into a plasmid and eventually inserted into bacteria through PCR. These bacteria deliver the transgenes into the plants which transform into Bt crops then. Another alternative also exists which uses gene guns for insertion of the plasmids into the bacteria.

From the conducted interviews with the three experts (Dr. Monika Messmer, Dr. Ciro Antonio Rosolem, and Mariana Gomes), we found out how they perceive Bt cotton in the market. As far as we can tell, all of them seem to approve of the technique and its application. They foresee a future in Bt cotton throughput the world and think it increases sustainability.

In short, Bt cotton seeds have been an economic success and researchers are aiming to find new, more efficient ways of acquiring Bt cotton seeds for more substantial commercial use. Modern engineering techniques, such as CRISPR/Cas9 systems, facilitated biotechnology by enabling to alter the plant's genome through simulated point mutations directly.

All in all, the entrepreneurs gain more profit than farmers, making the entrepreneurs the winners and the farmers the losers. With the use of GM cotton, there are no longer diseases or other health impacts, avoiding pesticidal or insecticidal infection. The farmers might not have physical problems anymore, but psychical issues, which may lead to risen farmer suicide.

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