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Terminator gene technology – their mechanism and consequences

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Abstract

The terminator technology is a genetically engineered suicide mechanism that can be triggered off by specific external stimuli. The preferred trigger is antibiotic tetracycline, which is applied to seeds. As a result of which the seeds of the next generation will self-destruct by auto-poisoning. The main version of the terminator includes a set of three novel genes inserted into one plant. However, there is another version, which divides two or three genes on to two plants that are later to be cross-pollinated. The ultimate outcome is a dead seed in the following generation. Many consider terminator technology a problem due to the fact that the top 10 largest seed companies globally control half the world's commercial seed market. Therefore, if terminator technology is commercialized, corporations will most likely try to incorporate this technology into all of their seeds. This would secure a much stronger monopoly on the seed market compared to patents because this technology would ensure that it is impossible for farmers to re-use their once harvested seeds.

Introduction

One of the biggest myths perpetuated by the advocates of modern biotechnology is that these technologies, and especially genetic engineering, are likely to provide a solution to world hunger. While technology brings relief to life's drudgery, it also carries social, economic and ecological costs. This side effect of technical development has become obvious with the advent of the green revolution, which has led to decrease in biodiversity and an increase in pesticides use. Biotechnology and genetic engineering are revealed as chemical free solutions to the problems created by the technology of the green revolution.

The terminator gene technology, or genetic use restriction technology (GURT), is the genetic modification of plants to make them produce sterile seeds in second generation which is also famous as a "suicide seeds". It is a biotechnological innovation patented in the United States that present great danger to agriculture and food security worldwide, particularly in developing countries. Scientists have questioned the technology on ethical as well as scientific grounds. This technology was patented by U.S. Department of Agriculture and the seed com-

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pany, Delta and Pine Land Company – a subsidiary of the seeds and agrochemical multinational Monsanto/American Home products. The technology has been appropriately named "Terminator" by the Canadian governmental organization, Rural Advancement Foundation International (RAFI), which has spearheaded an international campaign against it. Terminator alters the expression of certain genes in plants so that plants terminate their reproductive switch, about the embryo and make themselves sterile. Such plants then produce seed that cannot germinate. Monsanto described this technology as a "gene protection technology".

Mechanism of Terminator Gene Action

Operon concept was put forth by Jacob Monad in 1961, which explains the gene regulations in all organisms. Based on this mechanism of gene expression, this technology was developed. Three stretches of DNA, which carry genetic information, was introduced into the plant for this purpose. The first bit of DNA has a particular type of promoter. A promoter is a stretch of DNA where the process of converting a gene into a protein is initiated. The promoter used for the terminator genes would become active only in the later stages of seed maturation.

However if only this promoter and lethal gene were inserted into plants, then even the first generation seeds would not sprout. Having a 'blocking sequence' between the promoter and the lethal gene so that the latter it is prevented from being expressed. The second bit of DNA carries the gene for an enzyme called recombinase which is able to recognize the excision sequences and remove these, along with blocking sequence, from the first strip of DNA. The recombinase (repressor) gene is kept in control by another type of promoter. This promoter can be repressed – the recombinase enzyme will not then be produced if a particular protein is present. A gene on the third bit of DNA keeps producing the protein, which represses the promoter for recombinase.

Plant cells are genetically modified by introducing the strips of DNA and plants are regenerated through tissue culture methods. Since the promoter is active only during a certain stage of seed formation; the lethal gene has no chance of being expressed. When the first generation plants go about the business of producing seeds, the blocking sequence is firmly in place to prevent the lethal gene for being active. The first generation seeds are therefore formed without any trouble. When the first generation seed mature, they are exposed to a certain chemical. This chemical is able to repress the protein by third strip of DNA and prevent it from repressing the promoter attached to the recombinase enzyme. With this repression removed, the cells of the mature seed produce recombinase. The recombinase promptly removes the excision and blocking sequences in the first-strand of DNA.

Although the promoter and lethal gene are brought together, the lethal gene is not expressed because the promoter has been chosen to be active only at an earlier stage of seed development, which is not safely past. As a result, these seed can be sold to farmers, and germinate properly to produce healthy plants. However, these second generation plants carry the promoter and lethal gene bidding their time to spring into action. A time comes when the second-generation plants start producing seeds. At the stage when the promoter becomes active, the lethal gene springs to life and the chemicals it produces disrupts the process of seed formation. As a result the second generation seeds will not be fertile.

According to the patent document, the promoter becomes active only in late embryogenesis, virtually the last stage in seed formation after most other fruits and seed structures, but will not germinate if planted. An alternative technique suggested in the patent is to use a pair of genetically modified plants and then hybridized them. The first strip of DNA (carrying the promoter for expression in late embryogenesis, the excision sequences, the blocking sequences and the lethal gene) is introduced into a plant cell

and regenerated to produce one transgenic parent plant. In a similar fashion, another parent plant is genetically modified to carry a germination- specific promoter linked to the recombinase gene.

The crossing of the two plants produces plants, which inherit both strips of DNA. When the first generation seeds are planted, the recombination gene is activated during germination and removes the excision and blocking sequences from the first strip of DNA. As in the earlier case, the lethal gene becomes activated only when the plant tries to produce the second-generation seeds, which become sterile as a result. The promoter for the lethal gene; the patent points out that this promoter should not be a "leaky" one: it should be active substantially only during a well-define phase of plant growth or under particular environmental conditions, and should be inactive at all other times.

A promoter active in late embryogenesis, such as the LEA promoter, was ideal when the aim was to have a trait appear after the first generation. Such a promoter would be "active only after the first generation plant has completed a season of vegetative growth (embryogenesis is virtually the last stage in formation, after most other fruits and seed structure are formed)", the patent point out.

The Lethal Gene

A ribosomal inhibitor protein (RIP) gene is the lethal gene, the saponin 4 RIP being particularly preferred. RIP directly interferes in the expression of all proteins in a plant cell, without being toxic to other organisms. Expression of RIP in the cells of the embryo would be entirely preventing germination of the seed. The blocking sequence: when repressible promoter is used, the sequence which code for repressor can be used as a blocking sequence. When the blocking sequence is excised, the repressor gene is eliminated, thus further minimizing the chance of later inhibition of the system.

The Repressible Promoter

If it is sensitive to a chemical stimulus, the chemical should be nontoxic to the crop and to non-pest and not harmful to animals. The Tn10-encoded tet repressor-operator system, which is responsible to tetracycline, is preferred. Modified cauliflower mosaic virus 358 promoter containing one or more, preferably three, tet operons is used. The Tn10 tet repressor gene produces a repressor protein that binds to the tet operon and prevents the expression of the gene to which the promoter is linked. When tetracycline is present, it inhibits the binding of the Tn10 tet repressor to the tet operons, allowing free expression of the linked gene.

This system is preferred because the stimulus tetracycline is not to which the plants would normally be exposed, so its application can be controlled. Also, since tetracycline has no harmfully effect on plants or animals, its presence would not otherwise impede the normal development of the plant, and residual amounts left on the seed or plant after treatment would no significant environmental impact.

Recombinase and Excision Sequences

The recombinase-excision sequence system can be any one that selectively remove DNA in a plant genome. The excision sequences are preferably unique one in the plant so that unintended cleavage of the plant genome does not occur. A preferred system is the bacteriophage CRE/LOX system where the CRE protein preforms site-specific recombination of DNA at LOX sites.

The patent documents point out: "the present invention can be used to make a variety of transgenic plants. The method is particularly suited for use with plants that are planted as a yearly crop from seed. These include, but are not limited to, fiber crops such as cotton and flax; dicotyledonous seed crops such as soybean, sunflower and peanut; annul ornamental flower; monocotyledonous grain crop such as tobacco; vegetable crop such as lettuce, carrot, broccoli,

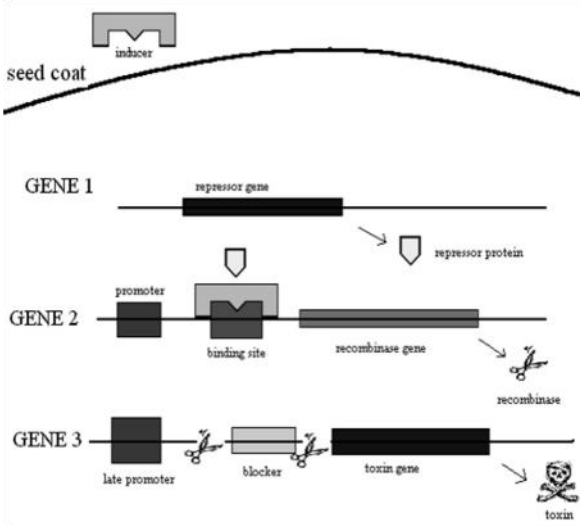

Figure 1. Mechanism of action of terminator gene after treating with inducer (tetracycline).
Source: http://cls.casa.colostate.edu/transgeniccrops/terminatordiagram.pdf

Terminator technology consists of three genes:
Gene I which produces a repressor protein that interacts with a binding site near Gene II.
Gene II is a recombinase gene that is controlled by a promoter. It produces a recombinase protein that snips out pieces of DNA.
Gene III produces a toxin that is lethal to embryos. It is controlled by a late promoter, which is active only when the embryo is developing. Between the late promoter and the toxin gene is a piece of DNA called a blocker, which interferes with the ability of the promoter to turn on the toxin gene.
There is a chemical called inducer used by the seed company that will initiate the terminator gene interactions. If the company does not want to initiate the terminator genes, it will not apply the inducer. This allows the repressor protein to bind on Gene II, preventing the production of recombinase. Then the blocker on Gene III is not snipped out, and the toxin is not produced. This allows the seed company to raise enough seed to sell to farmers. Before selling the seed the company applies the inducer. The inducer blocks the binding site on Gene II preventing the repressor protein to bind. Gene II then produces recombinase which snips out the blocker on Gene III. With the blocker removed, the late promoter is able to turn on production of the toxin gene late in the season.

cabbage, and cauliflower, and fruits crops such as tomato, zucchini, watermelon, cantaloupe and pumpkin" (Fig. 1).

Patent for Terminator Gene Technology

A biotechnological innovation was patented in the United States under Number 5, 723, 765 and titled "Control of plant gene expression" granted on 3 March 1998 by the United State patent office to the U.S. Department of Agriculture and the Seed company, Delta Pine Land Co., a subsiding of the seeds Argo chemicals multinational Monsanto/ American home products. Delta and Pine Land Company has been given exclusive license to sell the seeds, the USDA is to get a royalty of five per cent on the net sales. The USDA and Delta and Pine Land Company have applied for license in 78 countries. Delta and Pine Land Company is the largest cottonseed company in the world, and had a turnover of 183 million in 1997. Monsanto, its parents company operates in India in the seeds and Agro chemicals market. Monsanto which bought delta and pine land for terminator technology, is now largest pesticides firm, the second largest seed company and one of the 10 largest producer of pharmaceutical and veterinary medicine in the world.

International Property Protection of Seeds

The concern a raised from the proposal in the dunked draft on agriculture that seeds of new crop varieties would be treated as intellectual property and afforded protection in the form of industrial patents in a sid-generis form for granting of PBRS. These rights were introduced into Europe during 1960 to offer incentive to private seed companies to invest in plant breeding research. The legislation however made it clear that,

1. Farmers will have the right to save and replant their harvested seeds and they can

also sell portions of the seeds to a neighbor.
2. Other agricultural scientists can use the new variety as a parental line in any of their research programs.

With these two exemptions, PBRS, which have not been in operation in many countries for the past 30 years, have not created any serious problems either for the farmers or for scientists.

In USA Monsanto developed herbicide (ROUNDUP) resistant soybean variety. In 1997, about 13% of the commercially planted soybeans were the Monsanto is herbicide resistant soybean. Farmers using this variety have toagree in a contract not to save and replant the patented seeds. Only it can be used as food. Monsanto has kept strict vigilance over the contract farmer to protect violation of agreement.

Genesis of Terminator Gene Technology

Monopoly of MNC's in the Indian market

To prevent farmers from using seeds saved for sowing, particularly in the case of transgenic crops having desirable transgenic(s) for example 'Bt' for insect resistance, disease resistance and herbicide resistance, form this angle, the technology is quite relevant to pure lines open-pollinated varieties and hybrids. This technology will force the farmers to buy seeds fresh each year thereby increasing the turnover of the company. In fact, Monsanto of the U.S. that has developed the transgenic soybean seed with resistance to herbicides requires its customers to sign a license agreement ruling out the use of seeds obtained from transgenic crops.

The company has even employed investigators (private hired police) to identify unauthorized grower for facing legal action against them. This technology can also be used in higher yielding varieties developed by companies even if they don't have transgenes in order to increase the safety.

Protection of transgene(s)

Try to protect the transgenes(s) from being taken away by researchers elsewhere. The process of identification, isolation, modification and use of transgene(s) is time consuming and highly expensive. Once these organic plants reach to the farmers, plant breeders through hybridization can easily transfer the transgenes to a normal crop variety by backcross breeding.

This technology partly funded by USDA. USDA's goals to increase the valise of proprietary seed owned by US seed companies and to open new markets in second and third countries.It took four years band investment of USD 720,000 to develop this technique according to Rural Advancement Foundation International (RAFI). The USDA spent USD 190,000 and Delta and Pine Land Co. USD 275,000 on in-house research expenses and the two jointly spent USD 255,000.

Views of Farmer's Upon Terminator Gene

Farmer's organizations across India have demanded a ban on any seed material containing the terminator gene.

1. Questions on the ethical and social relevance of the technology in a country like India were farming in the major occupation.
2. There is the danger of this technology affecting the unintended targets, through pollen transfer.
3. The seed is used for consumption it may cause health hazards due to treatment of the seed with chemicals (or) toxins.
4. The country's rich genetic biodiversity will be lost. Maximum crop field may be covered by just one genotype, and it will eliminate the farm conservation traditions and location specific varieties.
5. The impact of tetracycline soaked seeds on soil ecology, particularly on microflora and fauna will be dangerous.

Seed Sterility on the International Agenda

International public organization such as FAO has discussed this issue at the meeting in May 1998 in the conference of parties (COPIV) to the convention on Biodiversity (CBD). The parties adopted a decision, which called for a precautionary approach to consider whether there are any consequences for the conversion and sustainable use of biological diversity from the development and use of the new technology. The subsidiary body on Scientific Technical and technological Advice (SBSTTA) is required to produce a back ground document based on which the secretariat of the GBD will come to official position.

Consultative group on international agricultural research which seeds as its mandate, supports the promotion of agriculture research for the poorest. CIGAR stated that transgenic crops could improve food yields by up to 25% in developing countries. A technology to produce sterile seed is also contradiction to the CGIAR'S efforts to transfer apomixis into crops. Apomixis is a type of asexual reproduction, which leads to offspring's genetically identified to the parental line. CIMMYT also developed a technology to transfer apomixis into hybrid maize. Farmers therefore could profit from the hybrid vague without purchasing seed yearly.

Ethical Aspects – Impact on Society

1. Terminator technology introduces in agriculture scenario will definitely favor large farmers and corporation over farmers and peasants.
2. It has negative impact on employment and consumers.
3. A very common underlying issue is that this technology debates lies the critique capitalism and particularly it monopolistic characterized by the dominance of large multinational corporations.
4. The terminator mechanism considered as a bias towards high tech type of agriculture of no relevance to problem of small peasant farmers.
5. Genetic resource have been regards first, as the common heritage of mankind due to this technology, biodiversity will be eroded quickly.

View of Different Organisations

Rural Advancement Foundation International

RAFI is non-governmental organization of North America describe this technology as "terminator technology" and "neutron bomb of agriculture".

This technology will terminate farmers and there after the food security of over a billion farmers in developing countries. Over all in these countries farm, saved seed accounts for an estimated 80% of the total seed requirement.

GRAIN (Genetic Resources Action International)

It is a European non-governmental organization viewed that biodiversity of the crops may be lost by these technologies. Farm seed saving is necessary for farmers to adopt the seeds to their own seeds, thereby generating and mulching biodiversity in their fields.

In the developing countries, it is a general practice that farmers somehow manage to buy small quantify of very costly seeds of high yielding varieties without standing performance in terms of agronomic trait they multiplying the seeds for next crop. But the seed companies did not accept this practice of farmers due to monetary reasons and hence they wanted to terminate the viability either in the second or subsequent generation.

It is evident that in case of hybrid variety seeds, the hybrid vigor can be fully explored in the generation. In subsequent generations, the hybrid technology "protection systems help insure that individuals and companies developing

new traits and technologies for commercial varieties have ability to turn fair return on their investment".

Action against Terminator Gene by Indian Government

The farmer should enjoy certain rights as a cultivar and as a conserve of the gene pool. The farmer as a cultivar should not only have the right to keep his own seeds conserving part of his crop for planning for the next, but should also have the right to limited sale of such seed in nearby areas. The National Biodiversity Act is to protect biodiversity as well as to regulate access to benefits. Realizing the seriousness of the problem, the ICAR and the DBT set up a monitoring the committee, which cause out with a plan of action to ensure that this lethal technology did not enter India. The government of India refused to the patent holders to sell the terminator seeds in the country. Impact of seeds containing terminator genes was also banned. The ICAR and DBT committee has recommended an institutional mechanism, including a legislative instrument and ask to made single point of entry for imported seeds and such other materials.

Overall disadvantages of terminator technology

1. Food and Agricultural Organization estimated that 1.4 billion poor farmers use farm saved seeds. In India 70% of the population involved in Agriculture and 90% of the farmers use farm saved seeds. But in USA 2% of the population of the population involved in agriculture. In India 30% of GNP contribute by the agriculture.
2. There is a danger of this technology affecting unintended targets. It is possible that these plants would transfer pollen to nearby wild type crops and cause sterility in their seeds too.
3. Harvested seeds are used only for consumption. It may cause health hazards for animals as well as human beings. Because it has been treated with some chemicals before sowing.
4. The country's rich genetic diversity will be lost.
5. Some genotype of a particular crop grown across the country leads to genetic vulnerability to pest and diseases.
6. Tetracycline is a chemical use to active the toxic gene, may alter the soil fauna and flora.
7. Location specific and season bound varieties cannot be grown.
8. Varieties of MNC's may not be suitable to all places and at as season different agro climatic conditions.

Advantages

1. This technology will induce private sector to make more investment in research and development of pure line varieties and open pollinated varieties because in these varieties the farmers do not change the seeds each years.
2. Farmers will use new seeds every year leads to maximum production.
3. This will result in stiff competition between the public and private sector institutions and ultimately the farmers will benefit through this technology.

Conclusion

Food is a big business all around world and multinational companies would only be happier if farmers from all around the world are forced to come back to them, year after year, to buy seeds. Ina country like India, agriculture research if it is to be relevant and realistic must be in collaboration with farms and farmer organizations and must be sensitive to the economic, social and conceptual framework within which farming communities work and make decisions. The terminator gene technology may be a good

one for American agriculturists because there are only two percent of people in this field. Here in India we have more than 75 percent of our population who are engaged in agriculture. Therefore, what is relevant to them may not be relevant to us. Hence, the research should not be completely business oriented but it must be service oriented so that findings will reach to the farming community in India.

REFERENCES

1. Bellows AC, Alcaraz GV & Hallman WK (2010). Gender and food, a study of attitudes in the USA towards organic, local, U.S. grown, and GM-free foods. *Appet J*, **55**, 540–550.
2. Bittman M (2011). Sustainable Farming Can Feed the World? Opinion – Opinionator. *New York Times*.
3. Crouch ML (2011). How the Terminator Terminates. The Edmonds Institute 1998.
4. Anon (2005). Genetically modified foods: breeding uncertainty. *Environ Health Persp*, **113**, A527–A533.
5. Haider R (2006). Biodiversity: Don't Sell "Suicide Seeds", Activists Warn. Inter Press Service News Agency.
6. Font MC, Gil JM & Traill WB (2008). Consumer acceptance, valuation of and attitudes towards genetically modified food: review and implications for food policy. *Food Pol*, **33**, 99–111.
7. "Moratorium". Ban Terminator. http://www.banterminator.org/Glossary/Moratorium Retrieved 12 December 2013.