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Terminator Technology: Comprehensive Understanding of Seed Suicidal Technology

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Abstract

In India, Plant breeding has been major concern of public sector institutes rather than profit oriented private seed companies for more than century. This was for protection of farming community and free flow of planting material among resource poor small and marginal farmers. Terminator technology provides legal right to the developers or originators of transgenic plants or animals to protect their material or breed from using by someone in an unauthorised way. The terminator technology prohibits the use of farm produced seed in the subsequent generation by the grower. Thus, farmers have to purchase fresh seeds at each season from the market. This technology not only restricts the use of seeds from the previous season but also ascertain monopoly in the seed industry.

Introduction

Terminator technology uses genetic manipulations to terminate the fertility or viability of stored seed and simultaneously restrict its reutilization. It is market driven technology in which the gene, involved in viability termination, called a terminator gene. The technique was noticed in public domain, when a patent (No. 57,23,765) was granted on "Control of Plant Gene Expression" at 3rd March, 1988 by the United States Patent and Trademark Office to the USDA (United States Department of Agriculture) and, the Delta and Pine Land Co., USA. The patent was granted to already known genes. These genes are responsible for the expression of desired traits either in the first generation of plant or in the subsequent generations.

Genetic Use Restriction Technology (GURT) refers to restriction of any genetic trait in a plant that can be switched on or off by the application of an external chemical inducer. The trait may include colour, softening, ripening, sterility, cold & drought tolerance etc. T-GURT (Traitor technology, trait-specific) refers to the restriction of a specific trait expression in a plant. V-GURT (Verminator technology, variety specific) refers to restriction of the variety by genetic engineering, plants whose seeds will not germinate if sown. The terminator technology uses a suitable lethal gene which makes the second generation seeds infertile or non-viable.

The first generation seeds (F_1) are, sold by the seed company, fully developed, normal and fertile to produce healthy plants bearing seeds or fruits that can be used as food but will not germinate if planted, which forces farmers to procure fresh seeds every year from the seed company, because they cannot use the harvested seeds of the previous year into the next season.

Genetic Basis of Terminator Technology

This technology employs three genes which carry the necessary genetic information into the plants.

1. Lethal Gene (Terminator)

Lethal gene produces a specific protein, toxic to plants and does not allow the seeds to germinate. A lethal gene encodes ribosome inhibiting protein (*RIP*), which interferes the synthesis of all proteins in the plant cells, without being toxic to other organisms. Thus, the germination of seeds would be inhibited by the expression of *RIP* gene in the plant cells' embryo. The *RIP* gene is attached with the specific promoter (LEA, Late embryogenesis abundance) which is activated only in the later stages of seed development. The LEA promoter was used to express the trait in the second generation onwards of seed (Figure 1A). The promoter is to be active only after the completion of vegetative growth in the first generation of the plant. A blocking sequence is placed in between the LEA promoter and the lethal gene to prevent the expression of lethal *RIP* gene in the first generation seeds. Specific excision sequence (LOX sites) flanks the blocking sequences. Site-specific excision excised out the blocking sequences at flanking LOX sites. Thus, the lethal gene comes in direct contact with the promoter and shows expression in all the subsequent generations during late embryogenesis stage.

2. Recombinase Gene

The second gene encodes an enzyme called recombinase. This enzyme recognizes the excision sequence (LOX site) and excised out these sequences along with the blocking sequence from the first gene construct by the recombination process. Bacteriophage CRE/LOX system is a preferred recombinase-excision system, where the CRE protein (Recombinase) performs site-specific recombination of DNA at LOX sites. Recombinase gene is placed adjacent behind to repressible promoter. This promoter is highly specific for a repressor protein encoded by the third gene. It can be repressed *i.e.,* recombinase enzyme will not produce if a particular repressible protein is present. The site-specific recombination (Figure 1B) takes place during germination of the first generation of seeds on sowing; and thus, removes the excision and blocking sequences from the first gene construct.

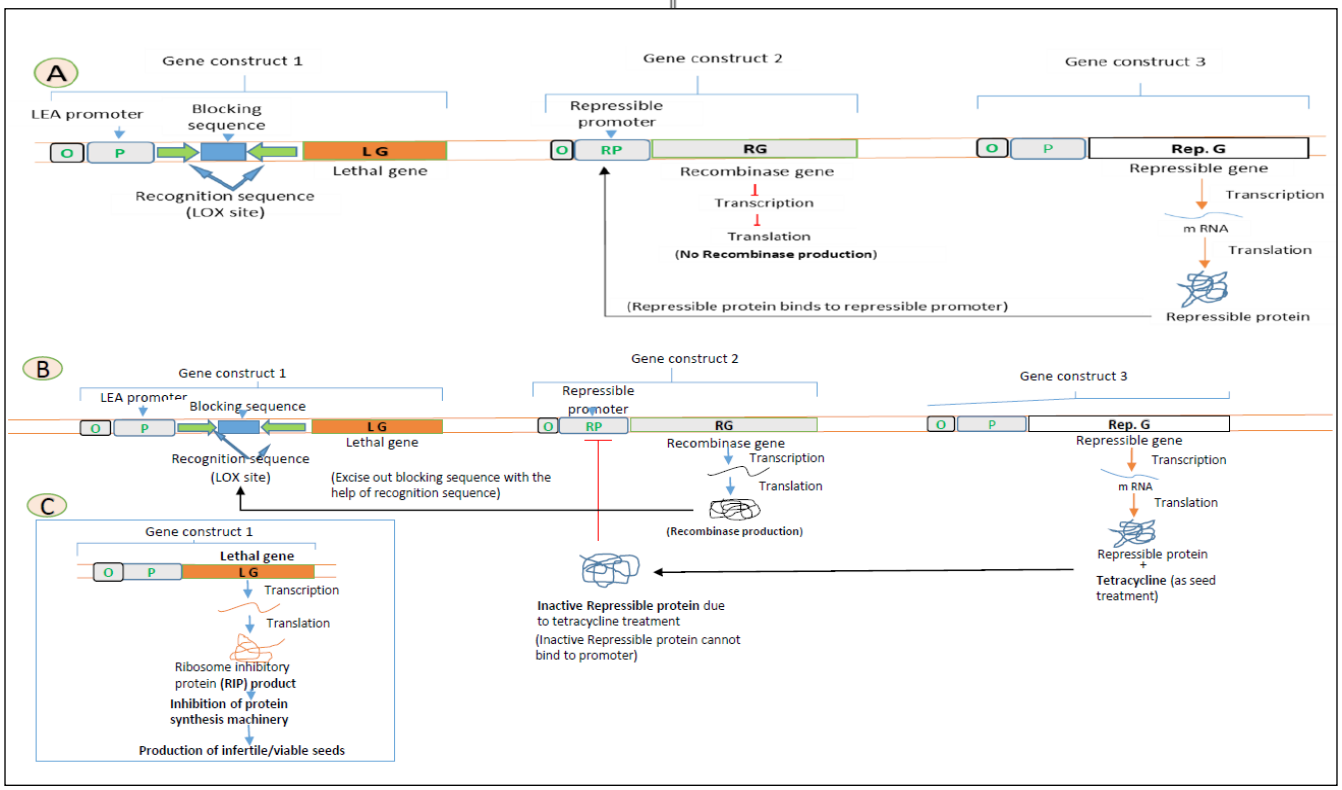

Figure 1 (A, B, C): Schematic representation of terminator technology, recognition sequence (LOX site) is excised out along with blocking sequence from first gene construct in presence of recombinase enzyme. Without its production blocking sequence remain intact in between of LEA promoter and lethal gene, thus seeds remain viable

3. Repressible Gene

The third gene, encodes a protein called a repressor protein, represses the promoter of the recombinase gene in the second gene construct (Figure 1C). The repressor protein itself becomes inactive when it binds to a specific chemical, *i.e.,* tetracycline. The inactive repressor (*i.e.,* repressor-tetracycline

complex) is not able to repress the promoter attached to the recombinase gene, thus allowing the synthesis of recombinase enzyme.

Use in Hybrid Seed Production

This technique is pertinent to only those crops which are cultivated through seeds only. Through biotechnological tools, genetically engineered parents (pure lines / inbreds) are being developed. One transgenic parent will contain gene construct having LEA promoter, excision sequence, blocking sequence, and lethal gene. Another transgenic parent will contain the gene construct having germination specific promoter and recombinase gene. Both transgenic parents are crossed to produce the F_1 (hybrid). Hybrid progeny contains both the gene construct from their transgenic parents. These F_1 hybrids are sown in the fields by the farmers. The hybrid seeds carrying germination specific promoter which will be activated during germination only. The activated recombinase gene will produce specific protein and will excise out the excision sequence and blocking sequence from the first gene construct. The hybrid plant will be heterotic *per se* and will produce a normal seed setting in terms of phenotypic appearance. The lethal gene will express itself during the sowing of seeds of the previous year into the next crop season *i.e.,* inhibition of germination. That is why farmers have to purchase new seeds every year from the seed company.

Use in Pure Line Seed Production

It is reported that plant cells will be genetically modified (transgenic) and the plants regenerated through tissue culture methods. During the first generation, *i.e.,* when companies are producing the seeds, the plants with these stretches of DNA will be normal. The blocking sequence is firmly present between the promoter and the lethal gene. Seeds are therefore formed without any trouble (Figure 2). When the first generation seeds mature, these seeds will be exposed to a certain chemical (tetracycline) and sold in the market to the farmers. The third gene produces a repressor protein but in the presence of tetracycline, repressor protein becomes inactive and cannot bind on the repressible promoter binding site. Recombinase gene will become active and removes the excision and blocking sequences from the first gene construct. At this stage, the LEA promoter is in direct contact with the lethal gene. However, the lethal gene will not express, because the promoter will become active only at a particular stage of seed development *i.e.,* late embryonic stage. As a result, the seed germinates properly to produce a healthy second-generation plant in the farmer's field.

When the second generation plant starts producing seeds, in the late embryogenesis stage, LEA promoter becomes active and produces a large amount of ribosome in activating proteins, which in turn inactivate the protein synthesizing machinery of cells, *i.e.,* ribosomes. This results in the production of non-fertile third-generation seeds. These seeds can be used as food, but will not germinate if planted to grow as subsequent generation plants.

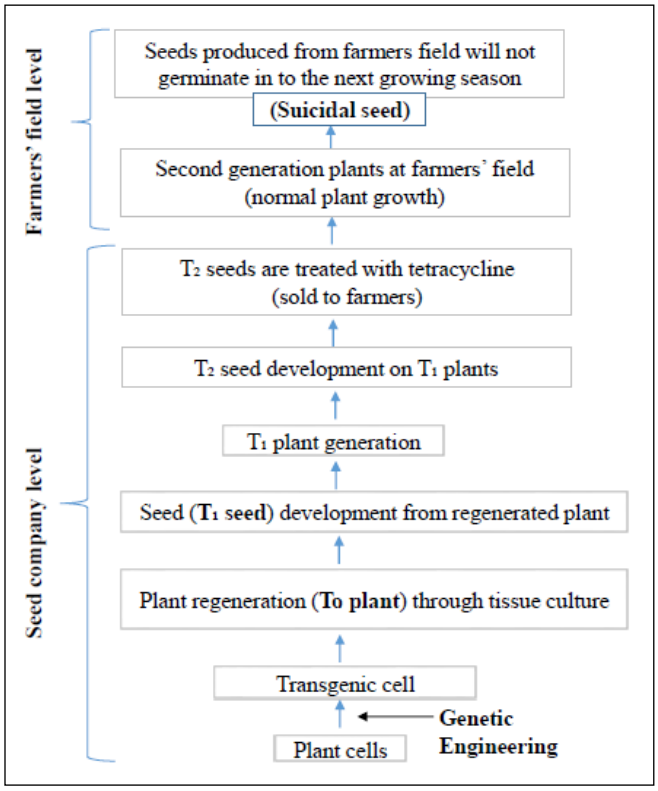

Figure 2: Schematic development of pure line terminator seeds through biotechnological tools by private seed companies and their response in farmers' field

Conclusion

The use of terminator technology can have diverse impact on users (farmers) and (developers) breeders. The GURT techniques provide special protection to the developers of transgenic material because material cannot be reused. This technology not only affects farmer's social status but also has great impact on agro biodiversity and environment.

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