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ISIS Report - July 12, 2001

Terminate the Terminators!

Terminator technology is a collection of genetic engineering tricks to make seeds sterile, so farmers cannot save and replant the seeds. The sole purpose of this technology, now owned by the big seed corporations in collusion with the US government, is to control seed production at source. It violates the basic human right of people to grow their food from saved seeds, and also introduce some of the most dangerous genes and constructs into crop-plants. This highly immoral and hazardous development must be stopped. All terminator crops that have been released commercially or undergoing field trials must be recalled and destroyed.

ISIS exposed the duplicity of biotech corporations that have been testing and growing terminator crops since 1990, while pretending that none has yet been produced. **Dr. Mae-Wan Ho** and **Prof. Joe Cummins** have written a primer explaining the technology in general terms (see "Terminator crops are here, ISIS exclusive" and "Killing fields near You", ISIS News 7/8). They have now written a sequel, which unravels several different versions of this deadly technology that have been patented (now available on ISIS website www.i-sis.org.uk).

- USDA and Delta and Pineland Company patent US5925808: Control of plant gene expression.
- Syngenta (Zeneca) patent US 5808034: Plant gene construct comprising male flower specific promote[r].
- United States Patent 5,750,867: Plant Genetic Systems (now Aventis): Maintenance of male-sterile plants.
- United States Patent 5,633,441: Plant Genetic Systems (now Aventis): Plants with genetic female sterility

The patents cover not only terminator techniques that engineer seed/pollen sterility, but also the control of expression of specific traits such as insect tolerance, drought tolerance or modification of secondary metabolism.

The overall aim is certainly to control either seed production or agronomically important traits at source. The genes used, as well as the constructs will have catastrophic consequences on biodiversity and health.

USDA and Delta and Pineland Company patent US5925808: Control of Plant Gene Expression, filed July 20, 1999/ Dec. 19, 1997

This is substantially the same as US 5723765, granted in 1998. It is a broad patent that includes not only constructs for controlling plant gene expression, but also the methods where by the transgenic plants are generated by transformation, the vectors for transformation, and the various crosses between plants. Cotton plants are mentioned specifically in this patent.

The main constructs are as follows,

1. A lethal, terminator gene, call it *gene a*, linked to a transiently active promoter, call it *p(t)*, the gene and promoter being separated by a blocking sequence, call it *block* flanked on either side by specific excision sequences, call them *ex*.

   p(t)-ex-block-ex-gene a

2. A second gene, call it *recom*, encoding a recombinase, specific for the excision sequence *ex* of the first construct, linked to a repressible promoter, call it *p(r)*, that is active during seed germination.

   p(r)-recom

3. A third gene, call it *repress*, encoding the repressor that binds to the repressible promoter *p(r)* to turn the second gene off. Although not mentioned in this patent, the repressor is one that can respond to an external chemical, such as tetracycline, which through a tetracycline responsive promoter *p(tet)* linked to the repressor, can turn the repressor on (or off, in another version).

   p(tet)-repress

In one version of how this is intended to work, The seeds are germinated, by the company, in the presence of tetracycline, which turns on the *repress* gene, the repressor protein binds to *p(r)* and stops *recom* from being expressed, so *gene a* is blocked, and nothing happens.

In the absence of tetracycline, say, when the farmer sows the seeds, the repressor protein is not expressed, so during seed germination, *recom* is turned on to make recombinase. The recombinase snips out the blocking sequence, *block*, and *gene a* is expressed. If *gene a* is a lethal gene that kills the male part of the flower, and *p(t)* is a promoter that acts only in the

male part of the flower, the plant will be male sterile. If *gene a* and its promoter are specific for the female part of the flower, the plant will be female sterile. If *gene a* and its promoter are specific for germination, then seeds will set, but they can't be resowed.

Each of the genes, promoters and repressors itself can be any one selected from an entire group of possibilities. Thus, *gene a* may be one of the following: lethal genes that kill the cell (terminator gene proper), insecticidal gene, fungistatic gene, fungicidal gene, bacteriocidal gene, drought resistance gene, protein gene product or a gene that alters secondary metabolism.

Similarly, the transiently active promoter *p(t)* may be a promoter that is active in late embryogenesis, in seed development, in flower development, leaf development, root development vascular tissue development, pollen development (male sterility), after wounding, during hot and cold stress, water stress, or during or after exposure to heavy metal.

The specific excision sequences and recombinase are selected from a group that comprise not only site-specific recombinase but transposase, flippase, resolvase, and integrase. Male sterility includes any lethal gene linked to an anther-specific promoter or pollen-specific promoter. Lethal genes include ribosomal inhibitor protein.

A lethal gene linked to a promoter that is active during late embryogenesis, for example, will give rise to seeds that are sterile, one that is linked to a promoter active during germination will result in seeds that fail to germinate. The blocking sequence can be a sequence that confers male sterility.

Syngenta (Zeneca) patent US 5808034:
Plant gene construct comprising male flower specific promote[r],
filed 15 September 1998).

This is an update on a patent first filed in 1990. It involves a cascade of gene regulation, the end result is the expression of a protein that disrupts pollen development. The disrupter protein is restricted to the male parts of the plant by an upstream promoter specific to male flowers. The male specific promoter being placed under the control of a regulatory sequence called the *operator*, that is turned off by a repressor protein binding specifically to it, and the expression of the repressor protein can be induced by a specific chemical externally applied.

The constructs are as follows:

1. A promoter *p(I)* responsive to the presence or absence of an exogenous chemical inducer, linked to the gene *repress* for the repressor protein.

   p(I)-repress

2. An operator *op* responsive to the repressor protein linked to the male specific promoter *p(m),* which is linked in turn to *disrupt*, the gene for the disrupter protein that kills the pollen.

op-p(m)-disrupt

When the chemical inducer is applied, the cascade goes as follows:

external chemical inducer Ý repressor Ý operator Ý no expression of disrupter protein.

The net result is the line can be maintained. In the absence of the external chemical inducer, the repressor is not expressed, so the disrupter protein is expressed and the result is male sterility. This is rather similar to the USDA-Delta Pine patent above, but the constructs are a bit simpler, and do not involve a recombinase.

Clearly the company can sell the proprietary chemical inducer to restore fertility to the line or to maintain it. As said, the whole point of the patent is to control the production of fertile seeds.

As in the USDA-Delta Pine patent above, each element in the patent can be realised with any one from a whole group of possibilities.

The chemical switch, *p(I)* , is exemplified by the promoter of the maize glutathione-S-transferase (GST II) gene, which is responsive to a host of chemicals, called ‘safeners’. Safeners, also known as antidotes are used to protect crops from herbicide injury, as they induce a family of enzymes, glutathione-S-transferases which catalyse the detoxification of a large range of hydrophobic (water-insoluble, fat-soluble) electrophilic (eletron-loving) compounds, ie herbicides, by joining up with them via the sulphydryl group, and causing their removal from the body of insects and mammals.

The patent lists many potential chemical inducers of the GST II gene . Safeners are used in combination with herbicides to reduce crop damage from the herbicide. The herbicide families requiring safeners are thiocarbamate and chloroacetanilide herbicides used to control weeds in corn, rice, sorgum and other grasses.

The chemicals listed in the patents include the safteners with common names like flurazole, napthinic anhydride, dicyclonon, oxabentrinil, fenclorim, cyometril, fluxofenim, furilazole and dietholate. There do not seem to be many publications reporting on the safety tests of the safeners.

United States Patent 5,750,867 (Plant Genetic Systems, now Aventis): Maintenance of male-sterile plants, filed May 12, 1998

This patent, first filed in 1992, covers "transgenic plants that have, *stably* integrated into their nuclear genome, a maintainer gene comprising a fertility-restorer gene and a pollen-lethality gene" (italics ours, because we don’t believe any evidence exists that the integrated foreign DNA is indeed stable). The plants can be used to maintain a homogeneous population of male-sterile plants. This specific patent covers maize plants, but the method has already been tried out in oilseed rape.

A complicated process for maintaining a male-sterile line is required, in which a male-sterile line is crossed with a 'maintainer line'. The male-sterile line is homozygous for a male-sterile gene, *barnase*, coding for a ribonuclease (barnase) from the bacterium *Bacillus amyloliguefaciens*, placed under an anther-specific promoter *p(a)*, *which acts early in the development of the male flower*. In other words, it has two copies of the construct, one on each of a pair of chromosomes. This can be represented as follows.

p(a) - barnase
p(a) - barnase

The 'maintainer line' is male fertile. It has the same genotype as the male-sterile line (ie, it is homozygous for the male sterile gene and stamen-specific promoter), and in addition, and not linked with the male-sterility gene, is heterozygous for a 'restorer gene' directed by a 'restorer promoter', *p(r)*, which is at least also expressed in the stamen cells, linked with a 'pollen-lethality' gene under the control of a pollen-specific promoter *p(m)*, ie, *one which is expressed late in the development of the male flower, in pollen-cells after* meiosis, the cell division leading to halving the chromosome complement. (The male-sterility gene is expressed before meiosis.) The restorer gene, *barstar* producing the protein barstar, also from *Bacillus amyloliguefaciens*, is a specific inhibitor of the male sterility gene product, barnase, while the 'pollen-lethality' gene, *lethal*, prevents pollen from being formed. The genotype of the maintainer line can be represented as follows.

Before meiosis

p(a) - barnase	*p(r) - barstar - p(m) - lethal*
p(a) - barnase	-

After meiosis

p(a) - barnase	*p(r) - barstar - p(m) - lethal*
p(a) - barnase	

As can be seen, after meiosis, the only viable pollen is the one with barnase, which confers male sterility. This pollen will spread the male-sterility trait around.

Each piece of the genetic jigsaw can be any one of several genes. Thus, *p(a)* can be the promoter of the *zm 13* gene from maize or the *TA29* gene from tobacco, or any promoter that directs expression in the tapetum cells of the stamen. The male-sterility gene *barnase*, can be any ribonuclease instead of barnase, while the restorer gene could be any ribonuclease-inhibitor instead of barstar, which is specific for the ribonuclease used as the male-sterility gene. The restorer promoter *p(r)* could be identical to *p(a)* so long as it leads to the expression of the restorer protein *at the same time and in the same cells* as the terminator protein.

As the restorer gene and the sterility gene are not linked, only half of the seeds of the male-sterile parent plants will be male-sterile, while the other half will have the same genotype as the 'maintainer line', unless the pollen containing the restorer gene is killed, and this is what actually happens. The pollen-lethal gene linked to the restorer gene prevents that male gametes containing the restorer gene from developing, so the only pollen available is one without the restorer gene, but carrying the male-sterility gene all the same (see diagram above).

When the maintainer line is selfed, the progeny will consist of 50% heterozygotes, and 50% male-sterile. So, it is necessary to incorporate a selectable marker, such as herbicide tolerance, next to the male-sterile gene or the restorer gene, or a different selectable marker can be put next to each. Say, a gene coding for phosphinothricin acetyl transferase (PAT) linked to the barnase gene in the male-sterile line, and that will enable only male-sterile seeds to be selected.

United States Patent 5,633,441 (Plant Genetic Systems, now Aventis): Plants with genetic female sterility, filed May 27, 1997

This patent, first filed in 1990, is similar to the male-sterile patent, except that a female-specific promoter is used to control expression of a lethal terminator gene in the female part of the flower without affecting the male part. The female-sterility gene is linked to a selectable marker gene with its own promoter, so that the female-sterile plants can be selected. In addition, a 'transit-peptide' is included in both the female-sterility gene and the marker gene to direct the gene product into chloroplasts or mitochondria, presumably so it does not affect pollen development, although many plants do have chloroplasts in pollen.

Terminator genes include, besides barnase, papain active protein, or the A-fragment of diphtheria toxin. Marker genes used include herbicide resistance gene, or a gene conferring a disease or pest resistance, a GUS gene for glucuronidase, or a gene encoding a *Bacillus thuringiensis* (Bt) endotoxin.

The second promoter (linked to the marker gene) may be a constitutive promoter (expression at all times in all cells), a wound-inducible promoter, a promoter which directs gene expression selectively in plant tissue having photosynthetic activity, or a promoter which directs gene expression selectively in leaf cells, petal cells or seed cells.

The patent claims include methods and vectors for making the transgenic plants, the various bits of DNA, the genes as well as the style-, stigma-, ovary-, seed- and embryo-specific promoters. Also claimed are the cell cultures, the hybrid seeds produced by crossing the female-sterile plant with a female-fertile plant; and a process for producing such hybrid seeds, as well as seedless fruit.

The plants for which the patent is claimed include corn, potato, tomato, oilseed rape or other Brassica species, alfalfa, sunflower, cotton, celery, soybean, tobacco, and sugarbeet.

Hazards galore

We have pointed out the hazards of terminator technology in earlier papers ("Terminator in new guises", ISIS News 3, December, 1999; "Killing fields near you", ISIS News7/8 Feb. 2001), and will only briefly recapitulate them here.

There are many different constructs, all of which have to be precisely engineered, and integrated into plants as intended, which is beyond the capability of current technology. A lot of gene scrambling occurs in artificial GM constructs as they are integrated, and genetic engineers cannot control where they are integrated either, thus multiplying the uncertainties and unpredictability of the GM crops produced.

The recombinase and similar enzymes in the USDA-Delta Pine patent is perhaps the most dangerous, as it is known to cause recombination at non-specific sites, thereby causing largescale genome scrambling (see "Terminator recombinase does scramble genomes", ISIS News 7/8).

The terminator lethal genes and gene products are known to be harmful to cells, including mammalian cells. Some of the most hazardous genes are designed to spread through pollen, including ‘male-sterile’ gene-constructs, and indeed, female-sterile constructs.

Genes and GM constructs can spread, not just through cross-pollination, but by horizontal gene transfer to unrelated species, and this process cannot be controlled. The instability of GM constructs in general and the complicated ones in terminator constructs in particular, increase the propensity for horizontal gene transfer and recombination. Horizontal gene transfer and recombination is one of the main routes for generating new viruses and bacteria that cause diseases, and for spreading drug and antibiotic resistance to make the diseases untreatable.

This highly hazardous and immoral development must be stopped, and all terminator crops that have been released commercially or undergoing field trials must be recalled and destroyed.

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