

V-GURT_s (Terminator) as a biological containment tool?

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(Tables removed from this edition)

Any biological containment system setting out to prevent gene flow of transgenes via pollen and seed must be 100% reliable and effective.

At present there are a number of molecular containment strategies that aim to restrict gene flow either via pollen, seed or sprouting of vegetative organs (e.g. tubers). Such strategies include male sterility, maternal inheritance, seed sterility, prevention of sprouting, apomixis and temporal and tissue specific control.

None of these containment systems claims to have the capacity to prevent gene flow both for pollen and seed except for V-GURT_s, also known as Terminator Technology.

V-GURT_s is a genetic use restriction technology that – in its design - uses complex inducible gene expression systems to make plants produce sterile seeds under specific conditions, i.e. induction.

As no field trials or green house trials, nor any details of a complete and fully functional V-GURT_s plant have been reported in the peer-reviewed literature, there is no scientific data available to analyse the performance of this technology and its reliability as a whole. Consequently a performance and risk analysis for V-GURT_s can only be carried out in part, focussing on the different individual genetic constructs and components or the different expression systems for which data is available.

The basic design of V-GURT_s as detailed in US patent 5,723,765 by the USDA and Delta & Pine Land is composed of 3 gene constructs, which code for

- a cell toxin expressed in the late embryonic stage that will result in sterile seed. The toxin gene is inactivated by a spacer (short sequence of DNA), which can be removed by a recombinase enzyme. To this effect, the spacer is framed by recombinase recognition sites (e.g. lox sites for the Cre recombinase).
- a recombinase enzyme that can activate the toxin gene,
- a repressor protein that blocks the recombinase gene unless an inducer is applied

Once the inducer (e.g. tetracycline) has been applied, it will remove the repressor protein from the promoter of the recombinase gene, thus recombinase is produced, which in turn will remove the spacer from the toxin gene. This now allows the expression of the toxin in the late embryonic stage of the seed, killing the cells and thus the growing seed.

There are other variants that use activators rather than repressors, but follow the same general principles outlined above.

The main components of V-GURT_s are thus a) an inactive gene coding for a cell toxin, b) an inducible site specific recombination system, c) an inducible expression system using external inducers and d) an external inducer.

General Problems

V-GURT_s is likely to only be as good as its weakest link. There are a number of known events which can interfere with reliable performance of any of the 4 components employed by V-GURT_s. Further research is required to address the following problems.

Gene Silencing and epigenetic changes to DNA:

Gene silencing and epigenetic changes to transgenes have been observed repeatedly in transgenic plants, especially under stress conditions (Broer 1996, Meza *et al.* 2001). RNA-mediated silencing and DNA methylation are considered to have evolved as part of a host defense mechanism, active against viruses and parasitic DNA which are active against transgenes (e.g. Riddihough and Pennisi 2001).

Recently, Srivastava and Ow (2003) for example, found that the site specific recombination system Cre/lox did not perform as expected and the authors suggest that the Cre gene underwent a genetic or epigenetic change.

Whilst gene silencing of the repressor gene would lead to sterile seeds, the silencing of either the recombinase or the toxin gene would result in viable seeds irrespective of whether the inducer was applied or not. The potential silencing of the late embryonic abundance (LEA) promoter, which drives the toxin gene, is regarded by Daniell (2002) as a crucial drawback of the terminator design as put forward by the USDA and Delta & Pine Land.

Reversibility of gene silencing (e.g. Mittelsten Scheid *et al.* 1998) may further add to unpredictabilities, e.g. seed sterility at a later stage.

Mutations:

Mutations frequently occur. Mutations of, for example, the introduced recognition sites (e.g. lox sites) would result in a permanently inactive toxin gene, leading to permanently viable seeds, i.e. in this and all subsequent generations. Equally, mutations to the recombinase promoter or gene would result in permanently viable seeds. Gene flow of the transgenes would thus take place.

Loss of promoter activity:

Loss or reduction of promoter activity over time has been observed in a number of genetically engineered systems. The observed loss of promoter activity in the tetracycline-inactivatable tTA expression system for example is presumed to be due to gene silencing (Tang *et al.* 2004).

Leaking promoter systems:

Many of the promoters in the inducible expression systems tested show a low level basal activity rather than zero basal activity. For example, leakiness of the tetracycline-inducible promoter system was reported by De Veylder *et al.* (2000).

Insufficient induction of promoter systems by inducing agent:

If the inducing agent, e.g. tetracycline, does not reach the target cells in sufficient quantity, the system will only be partially activated, resulting in viable seeds or in pollen capable of giving rise to viable seeds in neighbouring crops. As stated by Daniell (2002), "it will be difficult to ascertain whether all the seeds treated with the tetracycline inducer have triggered the gene switch (i.e. whether tetracycline has penetrated all the seeds.)"

Unspecific or unintended induction of promoter system:

Many inducible promoters can be induced by more than one agent or by plant endogenous chemical agents. The AlcR based ethanol inducible system for example can be inappropriately triggered by endogenously produced ethanol (due to anoxia) - (in Padidam 2003).

Segregation of the different genetic components during reproduction:

It is crucial that functional components of V-GURT systems and the introduced GM trait remain linked during reproduction. For example, if separation resulting from segregation should occur between the GM trait gene and the V-GURT genes, the GM trait may be passed on through seed and pollen to crops and weeds. Equally, if any of the genes involved in the V-GURT system were segregated from the others, the system would no longer function as required. To avoid segregation, V-GURT systems require that all the introduced genes be placed in very close proximity on the same chromosome (strand of DNA) to create a linkage that will reduce the likelihood of segregation as much as possible. If segregation occurs, any resulting gene flow of the transgenic trait (e.g. gene for Bt-endotoxin, pharmaceutical component or lignin reduction) to related cultivated, wild or weed relatives will occur and may be difficult to pick up in time to prevent it spreading more widely.

Conclusion

To date, no functional and complete V-GURT application has been detailed in the scientific literature. The evaluation of the capacity of V-GURT systems as a gene containment system presented here has thus relied on the evaluation of its envisaged components.

A system can be only as good as its weakest parts. At present, none of the components tested for any of the possible V-GURT systems are 100% reliable or effective. Given that at this stage the individual components of V-GURT systems offer less than 100% efficiency or reliability, the combination of these components in one organism will amount to still less. For example, if each of the 4 components performs to 95%, in combination their performance could reduce efficiency or reliability to as little as 81%.

Equally, future evolution of V-GURT systems must be taken into account. Because V-GURT systems confer an evolutionary

disadvantage selective pressure will favour genetic or epigenetic changes that lead to viable seeds or gene flow via pollen and capacity for reproduction, especially for example for transgenic trees. Effectiveness of V-GURT applications may thus decrease over time and generations.

A further drawback of V-GURT systems is that farmers growing conventional or traditional crops of the same species as the V-GURT variety in neighbouring fields will still find their crops contaminated by cross pollination. Whilst this is a problem for marketing their crops as GM free, especially if the GM crops in question were pharma crops, it may severely impact on food security. Farmers who save their traditional or conventional seeds for replanting may find a significant percentage do not germinate as a result of cross pollination, which in turn may lead to significant yield loss.

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