

GENES FROM A WILD PLANT *SOLANUM BULBOCASTANUM* USED TO RESIST POTATO BLIGHT FUNGUS

By Prof. Joe Cummins

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Biographical information

Joe Cummins is Emeritus Professor of Genetics, University of Western Ontario, London, Ontario, Canada. He is one of the earliest critics of genetic engineering.

He obtained his BS in Horticulture from Washington State University in 1955, and his PhD in Cellular Biology from the University of Wisconsin in 1962. He carried out postdoctoral research at the Universities of Edinburgh, Palermo, Stockholm (Karolinska Institutet) and at the Macardle Laboratory for Cancer Research at the University of Wisconsin. He taught genetics at Rutgers and the University of Washington, Seattle, before joining University of Western Ontario in 1972.

Since 1968 he been involved in environmental issues including mercury, asbestos, PCB and pesticide pollution along with waste sites and incinerators. His critiques of genetic modification began in 1988 when he encountered the power of multinational corporations over the Canadian federal government, and their refusal to face serious risk evaluations.

He has published over 200 scientific and popular articles, the most recent papers appearing in *Nature Biotechnology*, *The Ecologist*, and *Biotechnology and Development Review*. He is also a regular contributor to the Institute of Science in Society's website www.i-sis.org.uk and to its quarterly magazine, *Science in Society*.

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The German company BASF Plant Science GmbH is planning to test genetically modified (GM) potatoes by deliberately releasing them into the environment at Arodstown, Co. Meath, Ireland. The field trial is planned for the five year period 2006 - 2010.

The GM potatoes are modified using a gene from a wild Mexican plant, *Solanum bulbocastanum* (related to potato), along with marker genes including a gene for resistance to a herbicide. The potatoes are modified to be resistant to the fungus causing late blight disease

No environmental and health studies appear to be planned (1). Animal feeding studies on the GM potatoes do not appear to have been done.

Any environmental release of untested GM crops to the environment is unwise because the transfer of single genes even between related plants has resulted in unexpected toxicity of the transgenic gene products resulting from altered structure and immunogenicity of the modifying gene product (2). When single genes from plants such as a bean were used to modify another plant, the pea, it was assumed that such transfers could not produce toxic products in the plant being modified. However, the unexpected prevailed.

The people and wildlife of Ireland should not be exposed to inadequately tested genetic constructions.

Late potato blight is one of the most devastating plant diseases. It is caused by the fungus, *Phytophthora infestans*, a pathogen of the potato and, to a lesser degree, the tomato.

In the potato, *Solanum tuberosum*, there are four main dominant genes for resistance to blight infection, R1 through R4. An additional 7 genes were identified, 5 of which are alleles of the complex R3 locus (for a total of 11 dominant R genes).

Hybridization with wild Mexican species began in 1909 and continues to the present. However, in spite of constant effort the *P. infestans* fungus rapidly developed strains that overcame the genetic resistance.

Chemical fungicides have been developed to control blight but these also succumbed to the versatility of the fungus.

The fungus has two mating types (A1 and A2) both of which appeared first in Mexico, however, only the A1 mating type was present in European potatoes until 1978 when the A2 mating type appeared in Britain. The presence of the two mating types greatly enhances gene exchange leading to accelerated loss of genetic resistance and fungicide control (3,4).

Early resistant potatoes were obtained using true sexual hybridization with wild Mexican species, but the resistant strains soon succumbed to mutants of the blight fungus.

A wild Mexican plant, *Solanum bulbocastanum*, was stably resistant to blight but could not be sexually crossed with potatoes. A process called somatic hybridization was used to create sexual hybrids. Somatic hybridization includes fusing cells from cell cultures of *Solanum bulbocastanum* and potato; the fused cells contain nuclei of both potato and *Solanum bulbocastanum*. When the fused cells undergo mitosis the chromosomes of the two species are mixed and a single hybrid nucleus is formed in the cell. The cells can be cultured on solid media to form a solid callous (tumor) which, when treated with plant growth hormones, produces plantlets that produce flowering plants.

These somatic hybrids have irregular meiosis and irregular chromosome pairing, but relatively stable blight resistant lines can be obtained (5,6,7). Along with the 11 potato blight resistance genes that produce broad spectrum resistance which is very effective against blight, these include the gene RB (8) along with the genes Rpi-blb1 (9) and Rpi-blb 2 (10) which are active in both potato and tomato. The somatic hybrids are useful in identifying resistance genes and are transmitted into potato breeding lines by crossing. Nevertheless, genetic modification of potato breeding lines is presently preferred because resistance can be introduced into commercial lines with greater speed.

The BASF proposal for field testing GM potatoes (11) involves the use of two broad spectrum resistance genes, Rpi-blb1 and Rpi-blb2. These two genes have a structure associated with regulatory genes called nucleotide binding site-leucine rich repeat (NBS-LRR) class of regulatory proteins. Many disease resistance genes code for proteins of that class. Numerous plant NBS-LRR genes are present in the typical plant genome, each protein is specific for a particular pathogen signaling a defense response, frequently a localized plant cell death called a hypersensitive response. The C terminus of the protein containing LRR recognizes a ligand feature of a pathogen activating the NBS signaling module to initiate the defense response (12). The blight fungus suppresses the potato defense genes in sensitive plants but is thwarted by successful defense genes.

The NBS-LRR resistance genes in plants are localized in the cell cytoplasm and do not span the cell membrane but are activated by pathogen signals that penetrate the cell (13). The plant NBS-LRR proteins generally produce antibodies when injected into mammals but the modifications of the disease resistance proteins by glycosylation or myristylation which contribute to the immune response have not yet been studied.

The BASF proposal (11) indicates that the GMO potatoes being studied were transformed using two plasmids each containing copies of the *S. bulbocastanum* resistance genes Rpi-blb1 and Rpi-blb2 both of which contained an intron. The two genes were each driven by Rpi-blb 1 or 2 promoter including an intron as an enhancer and accompanied by a transcription terminator from Rpi-blb1 or 2.

The plasmids also contained a mutant acetohydroxy acid synthetase (ahas) gene from the tiny mustard plant *Arabidopsis* that conferred resistance to the herbicides of the imidazolines group (which are not approved for use on potatoes in Ireland).

The ahas gene was driven by the nopaline synthase gene promoter of *Agrobacterium* and its transcription was terminated using the nopaline terminator. The transformed potatoes are herbicide tolerant but the herbicide is only used during selection of transformed potato cells and not during cultivation of the potato. All GM lines intended for the release contain one or two copies of the plasmid inserts. Neither the resistance genes nor the ahas gene is expected to effect pollen or seed dispersal of the potato. The possibility that the GM potatoes will outcross to field potatoes was not expected to be effected by the genetic modifications.

Interestingly, the expression of the modifying genes was not studied under extreme conditions of stress such as drought, waterlogging, heat, cold, nitrogen excess or starvation in glasshouse experiments. In the past, GM crops have been tested under optimum conditions for growth prior to commercial or test release into real environments. Certainly, stress conditions may lead to unexpected toxicity in GM crops.

The BASF proposal (11) states that the resistance genes are not expected to exert any toxic, allergenic or harmful effects on human health arising from genetic modification. The genetic modifications are assumed to be safe because plants contain numerous NBS-LRR proteins and cultivated potatoes contain R genes from the wild species *S. demissum*.

These assumptions of safety are specious.

The *S. demissum* genes in commercial potatoes are NBS-LRR genes but are not from the broad spectrum NBS-LRR genes used in the BASF potatoes. Mainly, however, observed findings show that the transfer of genes between related species may actually lead to proteins with powerful (sometimes fatal) immune responses (2).

The procedure used to scan DNA sequences for epitope specifying codes for allergic responses (IgE) would overlook the powerful immune responses leading to fatal or near fatal inflammation. It is only sensible to test glasshouse-grown GM potatoes for not only allergenicity but **also** for inflammation before releasing the GM potatoes to the environment.

The immune response described in reference 2 was triggered **by** altered protein modification following transfer between species. However, little information is available on the modification of plant NBS-LRR genes. It seems a simple matter to conduct animal experiments on glasshouse-grown GM potatoes prior to their release into the environment, yet

that does not seem to have been done. Risk assessments of the impact of the experiment on non-target organisms seems to be based on an assumption of safety, and do not provide for an adequate monitoring scheme. If the GM potato proves **to be** immunologically active, the impact on both human and animals may be severe.

In the BASF proposal the handling, release controls and disposal of *Pytophthora infestans* inocula and infected plants was alluded to but not described in detail. That should be done.

The isolation distance 20 meters to cultivated potatoes does not seem adequate. Control of GM seeds and tuber escape from the site did not seem to be adequately described.

Post-release treatment of the test site did not seem adequately monitored nor will it achieve a clean post-harvest site. There does not seem to be any reason that a round the clock guard cannot be kept over the test sites.

In conclusion, the considerations of human and environmental safety seem primarily based on wishful thinking rather than on any serious efforts to gather or obtain factual information on the safety of the GM constructs. Monitoring also seems based on wishful thinking rather than serious efforts to detect negative impacts.

BASF petitioned for field test release of resistant potatoes modified with Rbi-blb1 and 2 beginning in 2005 in the Netherlands. The notice of petition indicated that the GM potatoes would be released in Germany, United Kingdom and Sweden. (14).

In the United States five field tests have been undertaken using GM potatoes modified with RB1 and RB2 broad spectrum NBS-LRR blight resistance genes obtained from *Solanum bulbocastanum*. The releases were undertaken in Minnesota and Wisconsin, by USDA or the university of Minnesota (15). The isolation and deployment of the RB genes in potato has been described (16,17).

Field testing of broad spectrum NBS-LRR genes has begun with the potato blight resistant strains. Broad spectrum pest resistant strains of rice, maize, soybean, and numerous food crops will soon follow. It is imperative that the safety of these genetic modifications to humans and the environment be fully evaluated *before the GM crops are commercialized*. The proposition that the NBS-LRR family of plant pest resistance genes and their products provide safe transgenes for human consumption and for environmental release because they are found in food crops (and for that reason require no further testing) is simply foolhardy. The suggestion that NBS-LRR genes must be assumed safe until proven hazardous certainly appeals to greedy promoters of GM crops but does not serve the public good.

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