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No to Releases of Transgenic Plants with Antimicrobial Peptides

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Anti-microbial peptides provide the first line of defence against invading microbes in both plants and animals. The peptides are involved in innate immunity, and are 15 to 40 amino acids in length, most of them hydrophobic (water-hating) and cationic (positively charged). They provide protection from bacteria, fungi and viruses, acting mainly at the cell membrane of pathogens [1,2]. The peptides are beginning to be employed in medicine and in crop protection.

A synthetic peptide D4E1 based on the cecropin B peptide toxin (obtained from the moth, *Cecropia*), consists of a linear sequence of 17 amino acids: FKLRAKIKVRLRAKIKL (F for phenylalanine, K for lysine, L for leucine, R for arginine, A for alanine, I for isoleucine, V for valine). The peptide protected against *Aspergillus* and *Fusarium* fungi. It acted by binding to ergosterol, a sterol present in fungal cell walls [3]. On further tests, D4E1 was found to have broad-spectrum antimicrobial action, and was active against fungi belonging to the orders Ascomycete, Basidiomycete, Deuteromycete and Oomycetes, as well as bacterial pathogens *Psuedomonas* and *Xanthomonas* [4]. The D4E1 toxin also proved effective in the treatment of human *Chlamydia* infection [5].

Transgenic tobacco plants transformed with a gene for the peptide D4E1 (driven by a double CaMV promoter and terminated by the *nos* transcription terminator, accompanied by a kanamycin resistance marker) was resistant to fungal pathogens [6]. Poplar trees transformed

as in the transgenic tobacco was resistant to bacterial pathogens *A. tumefacians* and *X. populi* but not to the fungal pathogen *Hypoxylon mammatum* [7]. Cotton plants transformed similarly with the gene coding for D4E1 showed resistance to fungi including *Fusarium, Verticillium* and *Aspergillus*, hence the synthetic peptide was proposed to be effective against mycotoxin-causing fungal pathogens [8].

Field tests have been conducted on D4E1 transgenic plants in the United States, transgenic cotton in Arizona and California, and transgenic potatoes in Idaho [9]. It seems inevitable that petitions to remove the transgenic crops containing D4E1 from regulation are not far off. As the D4E1 gene and its peptide product are both fully synthetic, it will be a stretch to assume that the product is "substantially equivalent" to the natural product.

Meanwhile, researchers at the National Agricultural Research Center, Niigata, in Japan have created transgenic rice with genes of the antimicrobial peptide, defensin, from *Brassica*. The transgenic rice plants were resistant to rice blast disease caused by the fungus *Magnaporthe grisea*. The researchers went a step further and systematically altered the genetic code for defensin to produce synthetic peptides that were far more toxic to the fungus than the natural peptides [10]. Rice with the synthetic genes and peptides are being proposed for field-testing prior to commercial release in Japan, and little effort appears to have been devoted to evaluate the safety for human health and the environment.

We agree with microbiologist Dr. Takahiro Kanagawa, a senior scientist at one of Japan's leading research institutes that releases of transgenic plants containing anti-microbial peptides are dangerous [11]. Defensins and other peptides are, for plants and animals, their first defence against pathogens. Just as D4E1 was effective against *Clamydia* infections [5], alpha-defensins, identified in long-term nonprogressors with HIV-1 infection [12], may well have applications in preventing AIDS disease.

The danger highlighted by Dr. Kanagawa is that environmental releases of these antimicrobial peptides will lead to the evolution of resistance among microbial pathogens. As Dr. Kanagawa points out, there is already a report of yeast evolving resistance to defensin from Dahlia after two days of co-cultivation [13].

The evolution of resistance to antimicrobial peptides will severely compromise both the natural defence of the human immune system against disease and the possibilities of effective therapies emerging in the wake of the disaster of widespread antibiotic resistance.

As versions of the peptides also provide defence against pathogens in other animals and plants, the ecological impact of resistant pathogens could be devastating.

Another factor adding to the hazards to health and the environment is that the synthetic transgenes code for peptides that are significantly different from the natural versions. This may itself be responsible for toxic or other harmful effects that cannot be known unless thoroughly tested.

This article has been submitted on behalf of the Independent Science Panel to the Niigata Prefecture in Japan, in support of a legal action taken by 12 citizens seeking to halt the transgenic rice trial. Please circulate it widely and send it to your elected representatives. Submit it to the US EPA and demand comprehensive risk assessment for the transgenic plants producing antimicrobial peptides.

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