Biosafety Considerations for Genetically Engineered Rice

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Introduction

Rice feeds more than half of the world's population. In much of Asia, rice is the staple food. The highest producing countries of rice are in Asia; in 2003, China produced 166,417,000 Mt (metric tonnes) of rice paddy, India produced 132,013,000 Mt and Indonesia produced 52,078,832 Mt (FAOSTAT 2004).

Agricultural biotechnology has been developing at a rapid pace, and genetic engineering has been proposed as a means of improving various aspects of crop production. Rice has been no exception, and developing countries have been urged to facilitate the adoption of genetically engineered (GE) rice (Datta 2004).

Adoption of GE rice in Asia, particularly China, is seen as potentially demonstrating the benefits of genetic engineering and reducing opposition to it (Brookes and Barfoot 2004. China, the world's largest producer and consumer of rice, is reportedly on finalising the commercialization of the same (Jia 2004; Lei 2004). Chinese scientists have been researching GE rice since the 1980s. Research is also being conducted in other Asian countries, including Japan, India, the Philippines and Thailand.

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Despite the apparent positive outlook for GE rice, concerns have been raised in respect of its impact on the environment, human and animal health, and socio-economic situations (e.g. Cummins 2004; Stabinsky and Cotter 2004a, 2004b).

In particular, it appears that GE rice research has thus far, outpaced biosafety considerations. The Assistant Director-General for Asia-Pacific of the Food and Agriculture Organization of the United Nations (FAO), He Changchui, has been quoted as saying that Asian governments should move cautiously before approving commercial planting of GE rice (Mohanty 2004). He urged governments to undertake extensive risk assessment on food safety.

This paper examines some of the biosafety issues that will need to be considered before any commercialization of GE rice.

Research on GE rice

This section briefly and selectively highlights some of the research conducted on GE rice. Traits reportedly closest to commercialization are glyphosate and glufosinate tolerance, resistance to bacterial leaf blight (using the *Xa21* gene), and resistance to Lepidopteran insects (using Bt toxins) (Brookes and Barfoot 2004).

Herbicide tolerance

Aventis (formerly AgrEvo) has developed GE rice tolerant to the herbicide glufosinate ammonium. Two events, LLRICE06 and LLRICE62, are no longer considered regulated items in the U.S. and can be grown commercially (APHIS 1999). These GE rice have not been commercially grown yet, presumably due to the lack of markets. Bayer, which bought over Aventis, is currently seeking approval for the import of LLRICE62 for food, feed and industrial uses into the European Union (Bayer 2003). Monsanto is developing GE rice tolerant to the herbicide glyphosate, and has reportedly conducted field trials in Japan and the U.S. (Brookes and Barfoot 2004). Scientists have expressed various human cytochrome genes in GE rice, to confer tolerance to the sulphonylurea herbicides (Inui *et al.* 2001).

Insect resistance

The Cry toxin genes from the bacterium *Bacillus thuringiensis* (Bt) code for several insecticidal Bt toxins; these have been introduced into rice to protect against Lepidopteran pests, particularly yellow stem borer (*Scirpophaga incertulas*), striped stem borer (*Chilo suppressalis*) and rice leaf folder (*Cnaphalocrocis medinalis*) (Khanna and Raina 2002; Ye *et al.* 2001; Ye *et al.* 2003). The most frequently used Cry toxin genes are *Cry1Ab* and/or *Cry1Ac* genes (High *et al.* 2004).

Plant protease inhibitors like the cowpea trypsin inhibitor (CpTI) inhibit plant protein digestion in insects. The CpTI gene has been introduced into rice to protect against striped stem borer and pink stem borer (*Sesamia inferens*) (Xu *et al.* 1996).

GE rice with the snowdrop lectin *Galanthus nivalis* agglutinin (GNA) gene resists sap-sucking insects, such as the small brown planthopper (*Laodelphax striatellus*) (Sun *et al.* 2002). GE rice expressing three insecticidal genes (Bt genes *Cry1Ac* and *Cry2A*, and *gna*) provided protection against rice leaf folder, yellow stem borer and brown planthopper (*Nilaparvata lugens*) (Maqbool *et al.* 2001).

Disease resistance

Bacterial blight is caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The rice gene *Xa21* provides wide-spectrum resistance against *Xoo*, although the endogenous gene is expressed at low levels. Genetically engineering rice by inserting *Xa21* enhances bacterial blight resistance. *Xa21* has been pyramided (combining genes by conventional crossing) with a fused *Cry1Ab/Cry1Ac* gene to confer resistance to insects and bacterial blight (Jiang *et al.* 2004). Two transgenic lines, one with *Xa21*, the other with a rice chitinase gene for protection against sheath blight and a synthetic gene with fused *Cry1Ab/Cry1Ac*, were pyramided to resist bacterial blight, yellow stem borer and sheath blight (Datta *et al.* 2002).

Rice blast is caused by the fungus *Pyricularia oryzae*. A gene from a medicinal herb, *Trichosanthes kirilowii* expressed the protein trichosanthin in GE rice, delaying blast infection (Ming *et al.* 2000). Rice chitinase genes and maize genes triggering anthocyanin (a flavonoid pigment) production can also confer blast resistance (Brookes and Barfoot 2004; Gandikota *et al.* 2001).

Research on virus resistant GE rice includes resistance to rice yellow mottle virus (RYMV), rice hoja blanca virus (RHBV), rice tungro spherical virus (RTSV) and rice ragged stunt virus (RRSV) (Brookes and Barfoot 2004).

Tolerance to abiotic stress

GE rice has been developed to tolerate low iron availability in alkaline soils (Takahashi *et al.* 2001). Over-expressing a rice sodium antiporter (a pump that moves sodium ion) gene improved salt tolerance (Fukuda *et* *al*. 2004). Manipulating plant polyamine biosynthesis produced drought-tolerant rice (Capell *et al*. 2004) and the barley gene *Hva1* inserted into rice reduced drought damage (Babu *et al*. 2004).

Nutritional enhancement

Scientists have expressed provitamin A (beta-carotene) in rice grains, creating 'Golden Rice' (Ye *et al*. 2000), promoted as a cure for vitamin A deficiency (e.g. Potrykus 2003). GE rice rich in iron has been developed to combat iron deficiency anaemia. Insertion of a ferritin (an iron storage protein) gene from the bean *Phaseolus vulgaris* increased iron content up to twofold (Lucca *et al*. 2002).

Production of pharmaceuticals

Rice has been genetically engineered to produce pharmaceutical products. Field trials of GE rice that produce the human milk proteins lactoferrin, lysozyme and alpha-1-antitrypsin have been conducted in California since 1997 (Freese *et al*. 2004). In 2004, Ventria Bioscience proposed starting commercial cultivation of biopharmaceutical rice, but has been blocked by the authorities, for now.

Biosafety Considerations

There is a wide range of GE rice under development, offering potential benefits. However, all GE rice must undergo risk assessment. This section points to some general potential environmental, health and socio-economic impacts of GE rice.

Environmental concerns

Asia is the centre of origin for the genus *Oryza*. There are wild relatives of rice, known to hybridize with cultivated rice, and weedy relatives (e.g. red rice). Gene flow via outcrossing or cross-pollination is inevitable as the necessary spatial, temporal and biological conditions are met in many Asian rice-producing areas (Lu *et al*. 2003). Although outcrossing rates may be low as rice is largely self-pollinating, "given the vast area over which rice is cultivated and wild and weedy rices occur, transgenes will almost certainly escape into non-transgenic plants" (High *et al*. 2004).

Gene flow between cultivated rice (*O. sativa*) and the widely distributed wild rice *O. rufipogon* was shown to occur considerably under natural conditions (Lu *et al*. 2003). Gene flow was demonstrated with a noticeable frequency from cultivated rice to its weedy (~0.011-0.046 per cent) and wild (~1.21-2.19 per cent) relatives (Chen *et al*. 2004).

Weedy rice is already a problem in more than 50 countries in Asia, Africa and Latin America, reducing rice yield and quality. Traits such as herbicide tolerance, insect, virus and disease resistance, and abiotic stress tolerance, if acquired from GE rice by wild and weedy relatives, could significantly enhance their ecological fitness. One possible consequence is the creation of more aggressive weeds, with resulting unpredictable damage to local ecosystems. Chen *et al.* (2004) recommend that GE rice should not be released, when it has transgenes that can significantly enhance the ecological fitness of weedy rice or that confer herbicide tolerance, in regions where weedy rice is already abundant and causing problems.

Hybrids of GE rice and wild relatives could swamp populations of wild species, possibly leading to their extinction and impacting agrobiodiversity. Crop genetic diversity is important for food security, acting as a reservoir for future breeding efforts. As Asia is the centre of origin of rice, any release of GE rice there must be mindful of this fact. Traditional varieties of maize in Mexico, a centre of origin and diversity of maize, have already been contaminated by transgenes (CEC 2004; Quist and Chapela 2001). The Commission for Environmental Cooperation of North America (CEC) (2004) recommends strictly enforcing the current moratorium on commercial GE maize planting in Mexico.

Gene flow through horizontal gene transfer (HGT; no parent-to-offspring transfer of genes) from GE rice to soil microorganisms is an area of omitted research. However, studies have shown that HGT between GE plants and microbes occurs under certain conditions (Nielsen *et al.* 1998). Significantly, methods for monitoring HGT from GE crops to microbes are problematic and too insensitive to detect HGT (Heinemann and Traavik 2004; Neilsen and Townsend 2004). As such, even though monitoring so far has largely failed to observe HGT events in the field or has deemed frequencies too low or too rare to pose risks, claims that HGT is not a significant risk are not justified.

Widespread adoption of herbicide tolerant GE rice could lead to problems in the long-term. In the U.S., where GE crops have been planted commercially for nine years, pesticide use has increased overall (Benbrook 2004). This was primarily due to an increase in herbicide usage, largely because there has been a shift towards more herbicide tolerant weed species or the development of weeds resistant to herbicides, particularly glyphosate. This has led farmers to spray incrementally more herbicides, and ultimately would require the usage of more toxic herbicides.

The impacts of GE rice on biodiversity have yet to be adequately researched. Some herbicide tolerant crops (GE oilseed rape and beet) have significant effects on biodiversity (FSE 2003). Weed densities and biomass, and abundance of some invertebrates, were found to be lower in GE crops than in conventional controls.

Insects may eventually evolve resistance to insect resistant GE rice. If this happens, GE rice will no longer be effective at controlling insect pests and more harmful insecticides could be used instead. It is widely assumed that resistance to Bt crops will occur (Snow *et al.* 2004). In the U.S., there are strict requirements for planting Bt refuges (areas of non-Bt crops) to delay build-up of resistance. Such refuges may not be enforceable or practical on small farms like those in Asia, making insect resistance a real concern. It is also known that insects can adapt to protease inhibitors (Jongsma and Bolter 1997), so the effectiveness of CpTI in GE rice might be short-lived. Fungi, bacteria and viruses may also evolve resistance to GE rice resistant to them.

GE rice could impact non-target organisms (that are not direct targets of pest control), including beneficial species like natural enemies of pests (e.g. lacewings) and pollinators. Bt toxins have the potential to directly kill non-target insects (e.g. Losey *et al.* 1999). While pollen levels needs to be sufficiently high to cause acute toxicity, chronic effects at lower pollen levels cannot be dismissed. Tritrophic studies have shown increased mortality of non-target predatory lacewings when predating on herbivore insects feeding on Bt toxins and Bt plants (Hilbeck 2001). The effects of CpTI and GNA on non-target organisms have not been investigated fully yet and there is little experience with these GE crops. There is also little research on ecological consequences; as ecosystems are complex, impacts on one organism could have significant impacts elsewhere in the ecosystem (Snow *et al.* 2004).

Effects on soil biodiversity have not been adequately assessed. Bt toxin is released into the soil from roots and can accumulate in the soil, implying that soil organisms can be exposed to the toxin over a long time (Saxena *et al.* 2002). There are indications that earthworms are affected when fed Bt maize litter; after 200 days, the earthworms experienced significant weight loss (Zwahlen *et al.* 2003). Studies have identified changes in important biological activities when Bt rice straw was incorporated in water-flooded soils, indicating a probable shift in microbial populations or in metabolic activities (Wu *et al.* 2004).

Health concerns

It is now internationally recognised that genetic engineering can cause unintended effects, e.g. by the Codex Alimentarius Commission (joint WHO/FAO agency), which deals with the international regulation of food safety. Codex principles and guidelines related to risk analysis and food safety assessment of GE food (Codex 2003), adopted in 2003, clearly oblige an analysis of unintended effects, by requiring a case-by-case pre-market safety assessment that includes an evaluation of both direct and unintended effects that could result from gene insertion (Haslberger 2003).

Unintended effects can result from the random insertion of DNA sequences into the plant genome, which may disrupt or silence genes, activate silent genes, or modify gene expression. Insertion of transgenic DNA is often imprecise, and associated with significant rearrangement and/or loss of plant genomic DNA, as well as multiple copies, multiple insertion sites, multiple insertion of parts of the event and insertion of extraneous material, e.g. from the vector (Collonier *et al.* 2003; Wilson *et al.* 2004).

In all commercial GE plants that have been carefully analyzed so far, the transgenic inserts found in the plants are rearranged compared to the sequences first notified to regulators (Collonier *et al.* 2003). The nature of the rearrangements includes deletion, recombination, and tandem or inverted repeats. Moreover, rearranged fragments of the insert can be scattered in the genome. Some of the rearrangements involve the cauliflower mosaic virus (CaMV) 35S promoter, which has a recombination hotspot (Kohli *et al.* 1999). The CaMV promoter, used in some GE rice, may also carry specific risks (Cummins *et al.* 2000; Ho *et al.* 1999, 2000a, 2000b).

Recombination may occur between plasmids before or during transformation, or between plasmid and genomic DNA during or *after* transformation. Transgenic inserts appear to show a preference for mobile genetic elements such as retrotransposons and repeated sequences. Transgene insertions into, or close to, such elements may lead to altered spatial and temporal expression patterns of genes nearby. All this may have unpredictable effects on the long-term genetic stability of the GE plants, and on their nutritional value, allergenicity and toxicology.

As rice is a staple food in Asia, thorough risk assessments must be done on GE rice. The most relevant testing for unintended effects is a well-designed feeding trial of adequate duration, conducted using the actual GE plant or product (not bacterial surrogate products, as currently accepted by regulators). In spite of the obvious need, few studies investigating the effects of GE food/feed on animals or humans have been published in peer-reviewed journals (Domingo 2000). Most animal feeding studies conducted so far have been designed to show husbandry production differences between GE and non-GE crops. The few studies that have been designed to reveal physiological or pathological differences demonstrate a worrying trend (Pryme and Lembcke 2003): Studies conducted by industry find no differences, while studies by independent researchers show differences that merit immediate follow-up.

For example, young rats fed GE potatoes expressing GNA showed changes in their gastrointestinal tract (Ewen and Pusztai 1999). Crypt length in their jejunums was significantly greater. The findings are similar to research describing fine structural changes in the small intestine of mice fed Bt potatoes (Fares and El-Sayed 1998). In addition, the number of cells in the crypt and the mitotic rate (number of cells dividing) increased in the jejunum of rats fed GNA potatoes (Pusztai *et al.* 2003). The implications for GE rice with GNA or Bt toxins have not been explored.

The liver of mice fed glyphosate tolerant GE soya underwent significant modifications of some morphological features (Malatesta *et al.* 2002). The liver had irregularly-shaped nuclei, more nuclear pores and more irregular nucleoli, suggestive of increased metabolic rate. However, the mechanisms responsible remain unknown. Glyphosate tolerant GE rice should be investigated for such effects.

Other health concerns include toxicity and allergenicity of transgenic products. One particular aspect of GE rice is that fused, stacked or pyramided genes are increasingly used, although the full health implications have yet to be considered. At the very least, the toxicity of *each* transgenic toxin, *and* the combinations of toxins, must be risk assessed (Cummins 2004). Nutritionally enhanced GE rice also needs to be evaluated fully, as changes are being made directly to nutritional content.

Many transgenic proteins contain sequence similarities to known allergenic proteins (Kleter and Peijnenburg 2002), a first indication of potential allergenicity. Notably, Bt protoxin Cry1Ac, expressed in some GE rice lines, is a potent systemic and mucosal immunogen (invokes immune response) (Moreno-Fierros *et al.* 2000; Vázquez-Padrón *et al.* 1999). Immune response should be further investigated for being indicative of a potential allergic response.

The persistence and fate of DNA and proteins from GE crops have not been extensively studied. However, *in vivo* studies showed that Bt protein (Cry1Ab), as well as transgenic DNA from Bt maize (fragments of *Cry1Ab* gene), survived digestion in the gut of pigs (Chowdhury *et al.* 2003). Others found that a 1914-bp DNA fragment containing the entire coding region of the synthetic *Cry1Ab* gene was amplifiable from sheep rumen fluid sampled 5 hours after feeding maize grains and "may provide a source of transforming DNA in the rumen" (Dugan *et al.* 2003:159).

If transgenic DNA survives digestion, it may be available for HGT to gut bacteria. This could possibly create new disease-causing viruses and bacteria, and spread antibiotic resistance marker genes (ARMGs) to pathogenic bacteria, making infections harder to treat (Ho 2004). The use of ARMGs in many GE rice lines is a concern. European legislation (Directive 2001/18/EC) mandates the phase out of ARMGs in GE crops, particularly if they are in medical or veterinary use. In the only human study, research showed that transgenic DNA can survive digestion in the human stomach and small intestine, and provided evidence of pre-existing horizontal gene transfer from GE soya to gut bacteria (Netherwood *et al.* 2004).

GE rice producing pharmaceuticals is intended for use by drug companies or in industrial processes, and not for consumption. The compounds are often biologically active chemicals and are potentially toxic. Pharmaceutical production should not be conducted in food crops because of the high risk of contamination (Editorial 2004). Contamination could occur via gene flow, grain admixture or human error. In 2002, soybean and non-GE maize were contaminated with GE maize engineered to produce an experimental pig vaccine (APHIS 2002). The CEC (2004) recommends that maize genetically engineered to produce pharmaceuticals and industrial compounds should be prohibited in Mexico and that a similar ban should be considered in other countries; the same should apply to GE biopharmaceutical rice.

Socio-economic concerns

Rice is much more than a vital food crop; it is also culturally, religiously and socially embedded in many societies. For example, the Balinese have cultivated a diversity of traditional varieties of rice for religious ceremonies. *Subak* organizations, comprising rice farmers in adjacent fields, collectively irrigate the rice terraces. They also make decisions on all aspects of rice production, including of offerings at the small temple each *subak* has in the fields. These practices embody an agri-*culture*, intricately linking rice production with religion, culture and social relations. The potential contamination of traditional varieties of rice with transgenes from GE rice would be an affront to peoples for whom rice is, literally, life itself.

Contamination of non-GE rice could also jeopardize people's right to choose non-GE and could affect export markets. Some degree of cross-pollination of non-GE rice is almost inevitable. GE rice previously planted in the same field and seed in the soil seed bank could germinate at a later date, contaminating non-GE rice. Seed saving and seed exchange, common practices in Asia, and spillage during transport, could also lead to inadvertent spread of GE rice.

Another issue to consider is that of intellectual property rights (IPRs) over GE rice. Many patents on rice genes have been lodged; in 2001, 240 patents had been granted on rice, 60.8 per cent of which were corporate-owned (Madeley 2001). Patented GE rice owned by corporations would take control of rice out of the hands of local farming communities. Should patented GE rice contaminate non-GE rice, the implications for farmers who traditionally save and exchange seeds are unclear.

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