

# GE RICE - The Genetic Engineering of the World's Leading Staple Crop

# 1.0 Introduction

Rice is the world's most consumed staple food grain, with half the world's people depending on it. It is harvested on about 146 million hectares, representing 10 per cent of global arable land. The yield is reported as 535 million tons per year and 91 per cent is produced by Asian farmers, especially in China and India (55 per cent of the total)." [1] Rice is not just a daily source of calories – it is intrinsically linked to Asian lifestyles and heritage. Present indigenous and local varieties are the product of centuries of breeding and selection by farmers to produce rice suitable to their environment and needs.

With the advent of science and modern technology in agriculture, the arrival of uniform seeds, fertilizers, herbicides and pesticides, the rich diversity of rice varieties has decreased drastically. Whilst the Green Revolution opened a profitable market to chemical corporations, seeds remained largely in either the farmer's or the public's hands.

With about three billion people consuming rice, profits promise to be high for any company or corporation that can acquire proprietary rights over the rice seed. Genetic engineering is an important tool towards this end as it enables companies to claim ownership over the "new seeds" they have "invented" giving them a legal basis to control its sale and use.

The two traits used for this purpose are the same as used in corn, soya or cotton, namely herbicide tolerance and pest resistance, the latter through internal pesticide production. (i.e. through genetically engineering the plant to produce its own pesticide). Both these traits go hand in hand with large scale monoculture production, and are a further step—the so-called Gene Revolution—from the previous Green Revolution.

Whilst many varieties vanished from the farmers' fields during and after the Green Revolution—many of them lost for good—the Gene Revolution will lead to a yet further decline of farmers' varieties and farmers' control over their own seeds.

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# 2.0 Genes, Gene Markers and Genetic Engineering

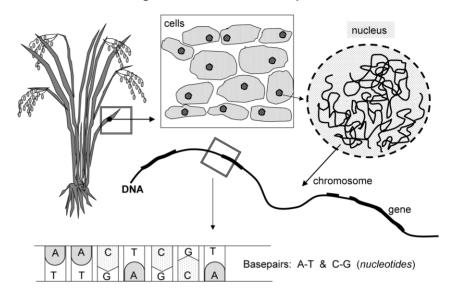
Rice varieties can be deliberately cross-bred so that the offspring will inherit a desired trait from one of the varieties, such as disease resistance, drought tolerance, or a specific flavour of the grains. This is nothing new - it has been going on for as long as humans have been farming. To obtain traits and characteristics from completely different species, such as bacteria, garlic plants or humans, is, however, a different matter. This is not a question of ordinary breeding skill, but of genetic engineering (GE), also known as genetic modification or manipulation (GM).

#### 2.1 Some Genetics

To understand how genetic engineering works, it is helpful to start by looking at a leaf, starting from the outside going inwards (see **Figure 1**). A wall-like structure composed of irregular cells can be seen. All plant parts—roots, stems, flowers or leaves—are made up of cells.

Inside each **cell** is a spherical structure called a "*nucleus*", the command centre of the cell. Inside the nucleus are a set number of "*chromosomes*", 'coloured bodies' that become visible when using a particular stain. *Chromosomes* are made up of "*DNA*"<sup>1</sup>, a long, thread-like molecule, which in itself is made up of four basic building blocks called "*nucleotides*" <sup>2</sup>—they are referred to as A, C, G and T—and

Figure 1. Inside the cell of a plant



together they form the *genetic code*. Arranged in *triplets*, i.e., three nucleotides at a time, information can be stored in 'coded form' along the DNA in a linear fashion, as if along a tape. The term 'DNA sequence' refers to this linear arrangement of the nucleotides and could for example read something like ACCGTAGGA.

The functions of all the DNA in a plant (or human for that matter) are not yet known. However, there are particular segments that are known as "genes". Genes are often referred to as the basic functional and physical unit of heredity. A gene holds the partial or basic instruction for the cell to make a particular product, usually a protein. The appearance, shape, activity or specificity of a protein is also dependent on other components and molecules in the cell, which can modify the protein. The nature of the product is defined by the DNA sequence of the gene. A gene can, for example, code for a structural protein with specific qualities such as forming a boundary between cells or providing movement, elasticity and the ability to contract. Different proteins can have different functions, such as digestion, breaking down of proteins or sugars, sexual regulation, transport of substances within the organism, disease resistance and fighting invading organisms.

The product of a gene is usually not needed all the time or by all the cells, but rather by a particular cell type at certain times, e.g., colour pigments in the cells of flower petals. or response to drought when there is no water. Therefore a gene has a particular structure or make-up that allows for finely tuned gene regulation, i.e., the gene can be switched on or off depending on demand. A gene is made up principally of three components: a regulatory element in the front called a promoter, followed by the coding sequence and finally another regulatory element called the terminal sequence.

Promoters are on/off switches (often finely tuned) that will respond to signals coming from the cell, the rest of the organism or even the exterior environment. Promoters can be development-specific, cell type-specific, and even species-specific. In general, promoters from rice or other plants cannot be used to regulate gene expression in bacteria or animals (e.g. insects, fish or humans), and vice versa. Clear exceptions are viral promoters. Viruses

DNA is the abbreviation of deoxyribo nucleic acid, a specific acidic material (molecule) that is present in the nucleus (thus called "nucleic acid". It is arranged as a double strand that twists in the shape of a "double helix". Protected by proteins, it is wound up and folded so as to safely store all the genetic information.

DNA is made up of four building blocks (nucleotides) which are: Adenosine (A), Cytidine (C), Guanosine (G) and Thymidine (T).

have evolved to invade cells and to command these host cells to reproduce new viruses by producing viral proteins and replicating the viral DNA (or RNA).<sup>3</sup> Thus promoters from plant viruses will work in most plants, sometimes even in animals and bacteria. The most widely used promoter in plant genetic engineering is the cauliflower mosaic virus 35S promoter (*CaMV 35S*), which works in all plants, but also in some cells of bacteria, fungi and animals.

The term *gene expression* refers to the activity of the gene when the genetic information is read and used by the cell.

# 2.2 Genetic Engineering (GE)

GE is the physical alteration or modification of the genetic information of an organism, using the tools of modern biotechnology. In particular, it refers to the introduction, elimination or rearrangement of specific genes or DNA sequences, using recombinant DNA techniques.

Genetic engineering enables the transfer of genetic information (DNA) across species boundaries. Genes taken from one organism and transferred to another are called *transgenes*. Theoretically any combination is possible, limited only by imagination! Examples include transferring spider genes into goats or fish genes into tomatoes. Rice, for example, has been genetically engineered with genes from many other species, including bacteria, virus, garlic, daffodil, insects and even humans.

Donors of transgenes may be organisms of another species, or may be from the same species as the

is DNA.

host organism. Transgenes may also be synthetically produced.

# 2.2.1 How is genetic engineering done?

- a) The target gene is isolated from the donor organism e.g., a Bttoxin gene from the soil-bacterium Bacillus thuringiensis (Bt). Initially, bacterial genes are easy to work with, as the genetic information that codes for a protein is present in one single block without any interruptions whereas genes of higher organisms (i.e. plants and animals) are more complex. Here the coding sequence is interrupted by a number of so called 'intervening sequences' or 'introns'. To obtain just the coding sequence thus requires extra steps and effort.
- b) A gene construct is made using: a) the coding sequence of the target gene - often modified so that it will work (or work better) in the host plant; b) a promoter that will work in the intended host plant, rice in this case; c) other regulatory sequences; and d) a "plasmid", a ring of DNA from bacteria which serves as the backbone and which allows for multiplication of the construct in bacterial cultures. If required, a marker gene, which might code for antibiotic resistance, herbicide tolerance or a fluorescence protein is included. The marker gene will later enable the 'marking' and thus recognition and selection of those plants or cells that have received the gene construct. Marker genes cannot be regarded as "neutral" or being "of no concern". If they remain in the plant, they are themselves "transgenes" and thus a safety concern and risk. A serious concern is that marker genes coding for antibiotic resistance may be taken up by gut bacteria, and
- ultimately be passed on to pathogenic bacteria. Antibiotics are crucial in human and animal medicine to fight many infectious diseases. The use of antibioticresistance marker genesgenes that confer resistance to antibiotics like hygromycin, ampicillin, neomycin and kanamycin—threatens to render these antibiotics useless as a treatment for diseases. Such a process of passing on genes and their traits from plant to bacteria or from bacteria to bacteria is known as "horizontal gene transfer". (See also horizontal gene transfer in section 4.)
- c) Getting the gene across: the process of transporting the gene construct through the plant cell wall, into the nucleus and inserted into the plant's own DNA is called transformation. The two main methods used to transform rice cells with a gene construct are: (a) the shotgun technique (or bio-ballistics), where thousands of copies of the gene construct are attached to tiny gold particles and literally shot into the plant cells (resulting usually in multiple copies per cell and extensive mutations [2]); or (b) using the infectious bacterium Agrobacterium tumefaciens as a shuttle to ferry the gene into the plant cells and into the nucleus. In both cases, single or multiple copies of the gene, or parts of it, or of the plasmid, will end up in the plant's DNA. In both cases, the location on the DNA where the gene construct gets inserted is completely random and the mechanisms by which the insertion works are not fully understood.
- d) Tissue culture and selection: Transformation is usually not carried out on whole plants but

RNA stands for ribo-nucleic acid and is very similar to DNA. It is also made up of four building blocks (here the nucleotides are called A, C, G and U). The threadlike molecule is usually only present in the form of a single strand, not in a double strand as

on individual plant cells, which are then grown into full plants. To turn plant tissue into individual cells capable of being transformed and then grown once more into full plants requires the use of chemicals and hormones. As a consequence, this method is *mutagenic* (causing genetic changes).

If the gene construct included a marker gene, plantlings (small plants) that have been transformed, i.e., that contain the gene construct, can easily be selected. If the marker gene, for example, was an antibiotic resistance gene, then the antibiotic is added to the growth medium that the plantlings are fed on. As small plants will die in the presence of antibiotics, only those that have the resistance gene will survive.

# 3.0 Conventional Breeding and Genetic Engineering (GE)

# 3.1 Breeding Methods Which Do Not Use GE

There are a number of ways to obtain new varieties and breeds, which may use genetics, or even biotechnology; but which do not use genetic engineering to move genetic material into the plant from an outside source.<sup>4</sup>

# 3.1.1 Traditional/Conventional breeding

This utilises the natural process of sexual reproduction usually only possible within the same species. Here the farmer/breeder takes two different parental varieties with specific traits and pollinates/fertilises one with the other, i.e., cross-breeds them. Seeds are harvested and sown, and the resulting plants are tested and selected for desired characteristics such as agricultural performance, nutrition, taste or cooking qualities. Traditional/conventional breeding is mainly based on trial and error and can be a long process that also depends on breeding skills.

# 3.1.2 Marker-assisted breeding or marker-assisted selection (MAS)

This is closely related to traditional/ conventional breeding. As above, two different parental varieties are cross-bred but the methodological difference is in the selection process. MAS is said to be much faster in achieving a particular trait than traditional breeding, but requires a laboratory. To detect whether a particular trait has been crossed from a parental variety into the new breed, a breeder can make use of known "markers" and use specific tools of modern biotechnology for detection purposes only, such as DNA sequencing. Genetic "markers" should not be confused with "marker genes" used in genetic engineering. "Marker genes" are added via genetic engineering whilst "genetic markers" are already present in the plant. Indeed, genetic markers function somewhat like fingerprints, they are distinct and particular DNA sequences that look different in each of the two parental lines. They are easy to detect with DNA testing methods. If the (approximate) location of the genes for a required trait on the chromosome is known, a distinctive genetic marker (fingerprint) from this region can be chosen - provided the sequence data is available for both parental lines, i.e. the trait and the nontrait lines. These are not markers that have been inserted, they are just short stretches of existing DNA that have a different sequence, "spelling" or "look" from the other parental line. With the help of laboratory tools one can for example, run a test that will check whether the DNA "spelling" is the one associated with the desired trait or with that of the "non-trait" plant.

The DNA test can be performed already at the seedling stage, thus telling the breeder early on which plants to select for further breeding. Although such biotechnological methods are being used for diagnostic purposes, they are distinctly different from plant genetic engineering because they are not being used to physically transfer new genetic material into the plant.

A recent success for marker assisted breeding in rice was reported in *Nature*, August 2006. Flood tolerance for a submergence period of 14 days was achieved by an international team, which had been able to identify genes crucial for flood tolerance in a rice variety and had introduced them into a widely-used Asian cultivar through breeding.[3]

#### 3.1.3 High-input varieties

These are also known as high-response or high-yielding varieties. Rather than resembling a breeding method, high-input varieties are a breeding aim or outcome. The methods used are either traditional/conventional breeding or marker assisted breeding.

As indicated by their names, these varieties have been bred and selected especially to respond to high inputs, i.e., agrochemicals and water. Again, this is done without genetic engineering. Yet whilst farmers' varieties are adapted to the soil, climate and other regional conditions, high-input varieties are bred and selected to produce high yields at any time and place as long as plenty of fertiliser is applied. On the other hand, if there are no chemical

<sup>4</sup> Genes or DNA transferred into a host plant/ species by means of genetic engineering can be derived from a species completely different from the host (here, the receiving plant) or from the same species as the host, or resemble an artificial design and be made synthetically in a test-tube.

inputs, such varieties usually perform rather badly, well below local or farmer varieties. Reports are increasing of high-yield rice varieties delivering poor harvests or declining yields despite applications of chemical fertilisers. The reasons for this are not fully understood. Farmers believe that using the same land year after year for intensive high-input agriculture has left the soil completely depleted of trace minerals. which are crucial to plant health and growth. Studies and reports of yield decline and soil depletion with highyield/input varieties have been published by IRRI as well as the FAO.5

### 3.1.4 F1 Hybrids

These are the first generation outcome of crossing two distinct varieties, i.e., pollinating one variety with the pollen of another variety. F1 Hybrids are of particular interest to the seed business because they give a uniform performance, while their harvested and replanted seed (F2) will not, thus discouraging seed saving. As a largely self-pollinating crop (up to 99%), rice has been a

5 See for example, the International Rice Commission Newsletter, 1997, Vol. 46 http:// www.fao.org/docrep/V6017T/ V6017T00.htm#P-1\_0; especially the case studies in section: Trends of yield and productivity of modern rice in irrigated rice systems in Asia http://www.fao.org/docrep/ V6017T/V6017T03.htm#P5\_1 and the section 'Scientific evidence of yield and productivity declines in irrigated rice systems of tropical Asia'. http://www.fao.org/ docrep/V6017T/V6017T02.htm#P5\_1

- <sup>6</sup> Arabidopsis thaliana (Thale cress) is a small plant often used in genetic research.
- Please note that there are two different types of tissue culture. It is the tissue culture type used in the transformation process that is highly mutagenic, not the tissue culture used for plant propagation. See Ref [2], Wilson et al. 2004, p21-22.
- 8 Some of these mutations are sometimes referred to as 'somato-clonal mutations' or mutations due to abnormal or inaccurate cell division.
- <sup>9</sup> Back-crossing: here crossing the GE plant with the non-GE parental line, then crossing the offspring back with the non-GE parental line, then crossing the resulting offspring with the non-GE parental line, and so on.

poor candidate for producing F1 hybrids on a commercial scale.

# 3.2 Creating Plant Varieties with Genetic Engineering

Genetically engineered (GE) rice is a rice variety in which the genetic information (DNA) has been deliberately modified by means of genetic engineering. GE means the addition of novel genetic material, most commonly obtained from completely unrelated species. In addition to containing novel genes and their products, such transgenes have the potential to interfere with or disrupt the expression of the plant's own genes. Furthermore, the processes involved in creating GE plants will alter the plant's own genetic information.

## 4.0 Problems with GE

Genetic engineering is distinctly different from the plant breeding methods detailed above. Novel genetic material is added and randomly inserted into the plant's own DNA. Along with the intended changes. such as herbicide resistance, genetic engineering also results in unintended changes, as detailed below. Both types of changes will often result in additional unpredictable effects and consequences. Furthermore, a GE plant will contain numerous mutations (genetic changes) as a direct result of the actual process of genetic engineering and transformation, also described below.

Unpredicted effects often cannot be easily explained or categorised, due to lack of information and understanding; to do so would require substantial further investigation. Bergelson from Chicago University, for example, described a case with clear relevance to rice. Her group was working with a plant called *A. thaliana*<sup>6</sup> (Thale cress), which, like rice, is highly selfing (self-pollinating). They engineered *A. thaliana* with a mutated gene from another

A. thaliana plant that coded for herbicide-tolerance. The result was not only a herbicide-tolerant plant, but the transgenic A. thaliana became, among other things, also 20 times more likely to cross-pollinate.[4] (See section on risks and impacts.)

# 4.1 Mutations Arising from the GE Process

The process itself of inserting the new genes into the DNA of plant cells (*transformation*) and growing these cells into plants via tissue culture<sup>7</sup> results in *mutations*, i.e., injuries and random alterations of the plant's own DNA.<sup>8</sup> Such mutations include deletions (loss of DNA), duplications, rearrangements, translocations and point mutations (mutations that affect just one single nucleotide).

DNA surrounding the insertion site of a transgene and the transgene itself can be badly affected by large mutations and chromosomal rearrangements (genome scrambling). Studies have further found that the whole of the plant's own DNA is affected and that the process of genetic engineering in itself causes hundreds to thousands of mutations per generated plant. [2, 5, 6].

Whilst the extent of mutations may be reduced by intensive back-crossing<sup>9</sup>, this is not being sufficiently carried out nor can it eliminate all mutations.

# 4.1.1 Consequences of mutations

In principle, any gene or regulatory sequence can be affected by transformation-induced mutations. This could lead in turn to the loss of gene function, altered protein function, and the loss or alteration of gene expression, e.g., expression in the wrong cell type or at the wrong time. Detection of such changes may not always be easy. For example, reduced nutrient levels, slightly enhanced

anti-nutrient levels,<sup>10</sup> and especially changes that become evident only under situations of stress such as drought or heat stress, are easily missed. [2, 7]

# 4.2 Gene Silencing

Gene silencing is a general term for a mechanism by which a cell can block the action of a particular gene or block the information encoded in a gene from being turned into a protein. It was first observed in field trials with 30,000 GE petunia plants, genetically engineered with the maize A1 gene<sup>11</sup> to produce red flowers. The regulatory sequence used was the '35S promoter' of the cauliflower mosaic virus.

Initially flowering red, the colour unexpectedly started changing during the growing season, with all shadings from red to white. [8] Upon investigation, researchers found that in many plants, the maize A1 gene was no longer working – it had been silenced. In fact, it was the regulatory 35S viral promoter sequence in front of the gene that had been shut down by the plant's own cellular system.

Since its discovery in 1992, the phenomenon of gene silencing is now frequently observed in genetically engineered plants, and especially under stress conditions. [9, 10]

10 An anti-nutrient is a substance with properties that interfere with the uptake or utilisation of nutrients; for example phytate or oxalate prevent calcium absorption, trypsin inhibitors prevent the proper digestion of protein.

Gene silencing can, for example, be evoked by: the insertion of DNA that is recognised by the plant as foreign (such as viral DNA), by multi-copy inserts of a transgene, or by a homology (sameness) between sequences of the transgene and the plant's own DNA. The onset of transgene silencing is often not immediate but can occur after a few generations of unaffected growth. It is heritable, but can also be revoked after generations.

# 4.2.1 Consequences of gene silencing

The above shows that the performance of the transgenes can become unreliable. This means that a GE trait which a farmer, processor or user relies upon can stop working within the crop without prior warning or indication, either resulting in reduced crop yield or value. In the course of transgene silencing, the plant's own genes can also become affected especially if there are homologies (areas of identical sequences) with the transgene or its promoter. Depending on which gene is silenced, i.e. which protein is blocked from being produced and the role of this protein in the metabolic pathways<sup>12</sup>, possible results for GE rice can be: lowered nutrient levels or enhanced levels of anti-nutrients, toxins or allergens. Other consequences can be weakened agricultural performance of rice, resulting in yield loss.

### 4.3 Positional Effects

Current genetic engineering methods and practices cannot predetermine the insertion site of a transgene. Where a transgene will end up in the plant's chromosomes is completely random and unpredictable. Accordingly, the impact and the consequences are also unpredictable. The position of the transgene will have an effect on the surrounding DNA

and genes, and vice versa. There are two scenarios to consider.

Firstly, the insertion of the transgene can disrupt a gene, a regulatory element (e.g. a promoter and enhancer<sup>13</sup>), or a regulatory region. The impact can be anything from deleterious to mildly disruptive and can affect plant health and performance, processing qualities, nutritional quality, allergenicity and toxicity. The impact of a gene insertion might not always be easy to measure or identify, especially as regulatory regions can regulate more than one gene, or regulatory elements can be situated far from coding regions.[11]

Secondly, the activity of the inserted transgene can affect the activity of other genes near the insertion site. This is particularly the case if the promoter (regulatory switch) of the transgene is a strong (or 'loud') promoter, which forces a constant high level expression of the gene. Such a promoter is, for example, the commonly used CaMV 35S promoter from the cauliflower mosaic virus. As a consequence of strong promoters and enhancers, the regulation and expression of nearby genes can become disturbed. Genes nearby have been found to become more active. Experiments carried out on transgenic rice plants and A. thaliana indicate that strong transgene regulatory elements can alter the expression of genes even from a considerable distance of several thousand nucleotides [12-14].

### 4.4 Pleiotropic Effects

Looking at its Greek origin, *pleio* means "many", and *tropo* means "changes". Pleiotropic effects mean multiple effects from a single gene, or a gene that is affecting more than one characteristic.

Identified unexpected changes include:

<sup>11</sup> The maize A1 gene encodes a 'dihydroflavonol reductase', which mediates pelargonidin pigmentation of the flower, conferring a salmon red or brick red flower colour.

Metabolic pathways: the cycles or chains of reactions occurring in living cells during which materials are broken down or built up with accompanying release or expenditure of energy.

<sup>13</sup> An "enhancer" is a regulatory DNA sequence that will collaborate with the promoter of a gene and enhance, i.e. magnify, the activity of the promoter, leading to a stronger gene expression. An enhancer does not need to be close to the promoter it collaborates with, but can do so from a significant distance.

- significantly increased levels of the cotton toxin, gossypol, in cotton seed harvested from Monsanto's Roundup Ready (RR) herbicide-resistant cotton [15];
- altered rotting characteristics in insect-protected Bt cotton [16];
- increased lignin production in some Bt maize [17] and in herbicide-resistant RR soya, the latter resulting in cracked stems in the summer heat which attracted fungal infection; [18] and
- a 68-fold reduced level of betacarotene (Pro-Vitamin A) and a four-fold increased sodium level in transgenic squash (CZW-3) approved for commercialisation in the US. [19]

### 4.5 Horizontal Gene Transfer

Horizontal gene transfer is a naturally occurring process that can be defined as the movement of genetic information (DNA) between cells and organisms by means other than sexual reproduction. This process enables the transfer of genetic information between sexually incompatible organisms and works across the boundaries of species, genera and even kingdoms, such as transfer of a gene from plant to bacteria.

Bacteria and fungi are able to take up DNA from their surroundings, whether this is from water, soil or gut. They can use this DNA as a food source or as genetic information, integrating it into their own DNA. The integration will usually only occur if there are DNA sections with clear sequence homology (similarities) between the new DNA and the organism's own DNA.

The transfer of GE genes (transgenes) from GE crops to bacteria or fungi in the soil, mouth or gut is hence a distinct possibility. It has been shown that plant DNA and genes can survive up to two years in soil, as well as being processed into food or feed. Transgenes as part

of plant DNA can also reach gut bacteria as they can survive digestion as shown for sheep [20] and also humans. [21] The transfer of the herbicide-tolerance gene from Monsanto's RR soya was reported by Netherwood in 2002. The UKbased research group carried out feeding trials on human volunteers (illeostomy patients after gut surgery) within a hospital setting. They found that the herbicide-tolerance gene (from GE soya) had survived digestion in the small intestine of all seven patients and furthermore, the transgene could be found in the micro-organisms of the gut in three out of the seven people. [22] Of particular concern here is the possible transfer of antibiotic-resistance genes from GE crops to gut bacteria, thus rendering certain antibiotics useless if used as medication.14

# 4.6 Overview of Risks Arising from GE

As previously explained, genetic engineering is distinctly different from conventional breeding. Furthermore, there is still a substantial lack of knowledge combined with scientific uncertainties. Problems associated and experienced with GE include mutations, gene silencing, positional effects, pleiotropic effects and increased potential for horizontal gene transfer.

To understand where risks arise, what they are and who or what would be exposed to them, one needs to understand what can be predicted and what cannot, as well as the potential hazards and the likelihood of their occurring.<sup>15</sup>

- a) The transgene can bring about intended and unintended changes, and both have predictable as well as unpredictable outcomes and consequences.
- b) The genetic engineering processby itself will create unintended

- changes with largely unpredictable outcomes and consequences.
- c) The transgene may be silenced. Again, with the desired trait ceasing to be active, there will be predictable and unpredictable consequences.
- d) Indirect risks and hazards need to be considered, such as the impact of repeated and broad scale spraying with broad-spectrum herbicides, such as glyphosate or glufosinate in the case of herbicide-tolerant crops, in this case, rice.

The areas that require special attention when looking at risks arising from GE rice are: health (food and feed safety), biodiversity and environment, agro-biodiversity, farming, socio-economics, loss of livelihoods, food security, food sovereignty, and farmers' rights. A big problem with GE crops and their use in agriculture is that once grown in the open, their traits cannot be contained, in fact, out-crossing and unintended mixing will lead to the GE contamination of conventional crops.

e) Out-crossing will occur if GE crops are grown within the cross-pollination range of sexually compatible plants, including conventional crops, landraces<sup>16</sup>, and wild and weed relatives. Out-crossing can have predictable as well as unpredictable outcomes and consequences. In general, the observed out-crossing rate of

<sup>14</sup> Antibiotic-resistance genes—originally taken from bacteria—have been repeatedly used as selective marker genes in GE crops to detect which plant or cells have successfully been genetically engineered.

<sup>15</sup> Risk can be defined as the gravity of a (potential) hazard multiplied by the likelihood of it occurring.

<sup>16</sup> Landraces refer to plants that are well suited and adapted to the environment in which they live, including to biotic and abiotic stresses.

Table 1. An overview of developments in GE rice

GE Rice Crops	Research	Development	Field Trials	Seeking Approval	Approved commercially	Commercially grown	
Agricultural Traits	Herbicide tolerance	LL rice	USA, (Japan)	Brazil Philippines*, Australia*, South Africa*, NZ*	USA Canada*, EU*	nil	
		RR rice	USA, Japan				
	Insect resistance	Bt rice India, China, Pakistan, Iran China, Iran, (India)					
	Lectins	□ GNA □□	r akistan, man				
	Proteinase inhibitor	СрТІ	China				
	Disease resistance	BB rice	Philippines, China	China ?			
	Stress tolerance		China				
Nutrition related traits	Pro-vitamin A	itamin A Golden Rice USA (2004) [India, Bangladesh - planned]					
	Iron enriched					nil	
Biofortification	Low gluterin		Japan (Aventis & J	lapan Tobacco - abandon	ed)		
	Tryptophan		☐ Japan				
PharmaCrops	Human lactoferrin	USA, Japan		USA (Ventria Bios	cience)	11	
	Human lysozyme	USA, Japan		USA (Ventria Bios	cience)	nil	
Industrial traits						nil	
Bio-remediation						nil	
Functional Gene Rice as Model S			Australia			nil	

rice is on average 1%, which has been high enough to lead to the development of thousands of different lines of rice during centuries of rice cultivation. The 1% out-crossing rate has also been sufficient to cause widespread GE contamination of conventional rice varieties and seed stocks in the US in 2006 and 2007.

f) The post-harvest mixing of grains between GE and non-GE varieties will occur, whether intentional or unintentional. Contamination of conventional maize, soya and oilseed by their GE counterparts have repeatedly occurred, and at times led to expensive recalls and compensation claims (e.g. the case of Starlink maize). Such accidental mixing can take place anywhere along the food chain between harvest and consumption.

# 5.0 GE Rice: Research, Development, Field Trials and Commercialisation

Rice is being genetically engineered in laboratories worldwide, by companies as well as public institutions. In some cases, genetic engineering of rice may be carried out merely to gain understanding of the function of particular genes and proteins and the processes they are involved in. Most GE however, is done for the purpose of application, i.e., to change or add a trait that is viewed as potentially useful, at least by those who perform or finance the research.

The research and development of GE rice can be divided into six areas, namely rice genetically engineered for (1) agronomic traits, (2) nutrition-related traits, (3) producing pharmaceutical compounds, (4) producing industrial compounds, (5) bio-remediation (the use of living

organisms to clean up toxins) purposes, or (6) research purposes only. Any distinction between nutrition-related traits and pharmaceutical traits are often blurred, although the testing required by law is usually much more stringent in the case of the latter.

If the progress is judged by the number of field trials conducted, developments for agronomic traits are the most advanced, followed by pharmaceutical traits and nutrition-related traits. Most of the field trials are carried out by companies and enterprises, yet they are hardly ever reported in scientific literature. Monsanto, for example, has conducted about 50 field trials with herbicide-tolerant RR rice in the US without making any report to the scientific community.

Of the 23 papers found in the scientific literature reporting on openair field trials, 22 were for agronomic traits and 1 for a nutrition-related trait. Of the 22 agronomic traits, 16 were for insect resistance (14 Bt and 2 proteinase inhibitor), 1 for bacterial blight resistance, 4 for herbicide tolerance (glufosinate) and 1 for drought resistance.

To date, no GE rice has been grown on a commercial scale. An overview of the extent of GE rice developments is given in Table 1. Details and specifics of developments are given below.

In this context, it is important to remember that health and biosafety aspects are not an initial concern in research and development. Thus a GE plant might produce or display a desired trait whereupon researchers and/or companies in search of publicity inform the press, but this does not mean safe and reliable use at a later stage is guaranteed or even possible.

# 5.1 Agronomic Traits

These traits are intended to alter the performance of the crop in the field. This category contains the vast majority of all developments and field trials of GE rice. It covers tolerance or resistance to so-called "biotic" stresses (weeds, pests, diseases) and "abiotic" stresses (e.g., drought, cold, salinity).

### 5.1.1 Insect/Pest resistance

The principle behind genetically engineered insect-resistance is to make the plant produce a substance that is lethal to the insect that is feeding on it. Usually, the gene construct is designed in such a way that this insect toxin will be produced in every part of the plant all the time. Whilst this might initially sound like a very good idea, it actually provides the perfect setting for insects to develop resistance to this insecticide. Due to co-evolutionary mechanisms, insects have the capacity to adapt to pesticides, no matter whether

these are externally applied or produced by the plant itself. Scientists largely agree that no GE pest-resistant plant grown on a commercial scale will be able to uphold its defence permanently, but rather that it will be a matter of time before it is eroded. Even plans to use *gene stacking* (or "pyramids"), i.e., to use a number of different genes in the same plant to provide a multi-layered defence, will not be able to maintain fields as "pest-free zones".

#### a) Bt toxins

The "Bt" in "Bt toxin" stands for *Bacillus thuringienses*, a soil bacterium. There are many different forms of and thus genes for Bt toxins—the most commonly used are Cry1Ab and Cry1Ac.

Bt toxins have been found to be particularly toxic to the insect order *Lepidoptera*, which are the butterflies and moths, whose larvae feed on particular plants and can—depending on numbers—become pests. Different forms of Bt are more toxic to one species than another. Genetic engineering with Bt toxins are mostly aimed against the yellow stem borer (YSB, *Scirpophaga incertulas*) and rice leaf folder (RLF, *Cnaphalocrocis medinalis*).

Field tests with Bt rice have been carried out in China (Cry1Ab, Cry1Ac, Cry1Ab/1Ac fusion, Cry1C, Cry2A), India (Cry1Ac – Monsanto's Bt rice), Pakistan (Cry1Ac & Cry2A), Iran (Cry1Ab), and Spain (Cy1Aa & Cry1B).

The use of Cry1Ac in food is highly problematic as it is regarded as a potent immunogen and can thus lead to allergic responses when eaten (details are given in the section on risks: health). For mode of action in insects and potential impacts on soil, see section on risks: biodiversity.

#### b) Lectins

Lectins are proteins that can bind or link to certain substances (carbohydrates) and in this way, become part of the defence system of an organism. Whilst considered to be the direct predecessors to the immune system, their role and function is not fully understood. It is known, however, that many plant lectins can be toxic when eaten by a range of organisms, including insects and humans.

High levels of lectins may be found in plants of the Nightshade family<sup>17</sup> (i.e. solanaceae or potato family), in grains or in beans. The snowdrop lectin GNA (Galanthus nivalis agglutinin) has been shown to be highly toxic to many insects. KV Rao and his team at the University of Hyderabad (Andra Pradesh, India) genetically engineered rice with the snowdrop lectin and reported high-level resistance to the whitebacked planthopper (Sogatella furcifera). [23] Saha et al. of the University of Calcutta, India, produced GE rice containing the garlic lectin ASAL. Greenhouse trials showed enhanced resistance to sap-sucking insects such as the brown planthopper and the green leafhopper. [24] Biotechnologists thus regard lectins "to be a potential component in insect-resistance rice breeding programmes." [24]

#### c) Proteinase inhibitors

Proteinase inhibitors (PIs) are a crucial plant defence mechanism. They are a particular category of proteins which when

<sup>17</sup> The solanaceae family is also known as the Nightshade or potato family. Members include potato, tobacco, tomato, aubergine (eggplant), capsicum (chilli pepper, paprika), mandrake and deadly nightshade (or belladonna).

eaten will inhibit, i.e. prevent, the digestion of proteins. As seeds and tubers are crucial for the survival of a species, their level of protective PIs is particularly high, between 1-10% of their total protein.

The main idea behind genetically engineering plants with particular PIs is to make every part of the plant indigestible to insects, not just seeds or tubers.

Rice has so far been genetically engineered with proteinase inhibitors including PIs from potato (US/China), maize (Japan), soybean (Korea) and cowpea (US/China/UK), the last including a field trial. Increased resistance was reported for brown planthopper (Nilaparvata lugens), rice weevil (Sitophilus zeamatis), pink stemborer (Sesamia inferens), striped stemborer (Chilo suppressalis).

Of the PI genes, the cowpea trypsin inhibitor gene (CpTI) and potato proteinase inhibitor II (PiNII) are at present the most commonly used in plant genetic engineering. As with lectins, biotechnologists suggest that IP genes "can be used as a general strategy for control of insect pests." [25]

Experiments have shown that the use of proteinase inhibitors as well as lectins may be toxic not only to the target organism (here, the crop pest), but also to harmless and even beneficial insects. Impacts on non-target organisms have a negative impact on biodiversity and sustainable agriculture, especially as crosspollination will carry the harmful pest resistance trait to farmers' varieties and wild relatives.

The potential implications for human and animal health are discussed in the section on risks.

#### 5.1.2 Disease Resistance

- a) Bacteria: Whilst there are rice strains resistant to bacterial blight (BB), efforts have been made to develop GE versions of bacterial blight-resistant rice. called BB rice. The genes used for this purpose are rice genes Xa21 and Xa26 taken from resistant rice. The notion that transferring a gene within the same species is equivalent to conventional breeding is false. As outlined in previous sections, genetic engineering processes and the random character of gene insertion give rise to mutations and unpredictable consequences (see section on risks). Recently, Swamy et al. (2006) performed an interesting experiment. Marker assisted breeding (which is not genetic engineering) was used to introduce three resistance genes into a blight susceptible rice (line MH2R).[26] A susceptible Pusa Basmati line was genetically engineered with the Xa21 resistance gene (PB1-Xa21). The GE rice line IR-Xa21 was also used. These three rice lines were exposed to 6 distinct Indian isolates (strains) of the BB pathogen, Xanthomonas oryzae. It is noteworthy that the genetically engineered PB1-Xa21 rice was susceptible to all 6 isolates, whilst the (non-GE) cross-bred variety had gained resistance. When comparing the results from genetic engineering with those from the non-GE marker assisted breeding, the authors came to the conclusion that breeding BB resistance is more effective than genetic engineering. The Xa21 gene was first isolated and used as a transgene by Californian scientists in 1995. [27]
- **b) Fungus:** Whilst there are conventional rice lines with partial or

- good resistance to fungal diseases, conventional breeding does not offer 100% protection. However, the genetic engineering approach fails to deliver a solution, despite major efforts to find candidate genes for resistance to the two main fungal pathogens, namely blast fungus (Magnaporthe grisea) and blight sheath fungus (Rhizoctania solani) that attack rice. [For review, see [28], esp. Table 3]
- c) Virus: Genetic engineering for virus resistance for rice has mostly focused on Tungro disease, with the two viruses involved being rice tungro bacilliformus virus (RTBV) and rice tungro spherical virus (RTSV). The common approach to engineer resistance to viruses has been to use sequences from the viral gene that code for the virus coat protein. Using this approach in rice, moderate tolerance ranging from 17% to 73% for RTSV was, for example, observed in greenhouse trials by an US/Malaysian team.[29] Whilst plants engineered in this way may exhibit increased tolerance, these may be short lived due to e.g. gene silencing. Furthermore, the use of viral transgenes can alter the behaviour and traits of invading viruses and "a single transgenic viral protein in a single host species may confer enhanced vulnerability to wellestablished viral diseases and it even may confer susceptibility to viruses which do not normally infect plants." 18 There are also reports of conventional strains of

<sup>&</sup>lt;sup>18</sup> Latham J and Wilson A. (2007). Transcomplementation and synergism in plants: implications for viral transgenes? *Molecular Plant Pathology* 8(6), 000–000 http://www.bioscienceresource.org/ commentaries/documents/BSR3-VirusTranscomplementation.pdf

- rice that are tolerant to RTBV, such as the Indonesian strain Balimau Putih. [30]
- d) Nematodes: Root knot nematodes (Meloidogyne species) have been reported as a problem in some areas. Genetic engineering approaches to counter insect pests, such as the Bt-toxin Cry1Ab, or the cysteine proteinase inhibitor as well as some lectins, seem also to have nematocidal properties, as tested with GE potatoes (briefly reviewed in [31]). There are likely to be impacts though on harmless nematodes and other soil dwelling organisms with potential negative secondary effects on the soil ecosystem and the interaction between the crop plant and soil organisms.

#### 5.1.3 Herbicide Tolerance

This approach defines any plant other than the GE crop as an unwanted weed and competitor. Plants have been engineered with bacterial genes to be tolerant to broadspectrum herbicides such as glufosinate or glyphosate-based herbicides. These herbicides must be applied in order to "benefit" from the genetically engineered trait.

# a) Glufosinate tolerance: Glufosinate is also known as phosphinothricin. The main glufosinate-based herbicides are Basta or Liberty, produced by Bayer. Bayer's herbicide-tolerant GE crops are thus known as LLcrops, with LL standing for Liberty Link. (The main transgene is the bar gene derived from the soil bacterium Streptomyces hygroscopicus, that confers resistance to the broad-spectrum antibiotic action of glufosinate). LL rice has been grown in about 80 field trials in the USA between 1996 and 2006. Three lines were tested, namely LL-Rice 62, LL-

Rice 06 and LL-Rice 601. Lines 62 and 06 received approval for planting and selling for human and animal consumption in 2002. Line 601 ceased to be tested in 2001 and was never put forward for approval. Yet it managed to severely contaminate conventional rice in the US in 2006, and the problem continues. After the extent of the contamination became evident. Baver put LL601 forward for approval (or "deregulation" as it is known in the US) and this was granted at the end of 2006.

- b) Glyphosate tolerance: The broad-spectrum herbicide behind glyphosate is Roundup, manufactured by Monsanto. The gene used to confer tolerance to glyphosate is a bacterial EPSPS<sup>19</sup> gene. Crops resistant to Roundup are called Roundup Ready, or RR. Monsanto has developed and carried out field trials of RR-rice in the US since 1998. Field trials have also been carried out in Japan.
- c) Resistance to abiotic stresses

Abiotic stress refers to any pressure or stress that is exerted not by living organisms, such as pests or weeds, but by the 'nonliving' environment; e.g., salinity, drought, flooding, extreme temperatures, and light conditions. Mechanisms of coping with such stresses are complex, and not down to the action of a single gene. Rather they require several genes interacting together in order for the trait to be realised. Often the same gene will be involved in a number of stress responses and yet depend on the activity of others for an effective response.

Genes involved in stress responses will also usually be part of the expression of other traits and mechanisms in the plant, and should thus not be viewed only as "stress response genes". Attempts to genetically engineer stress resistance (such as drought resistance) tend to focus on the transfer of a single gene. Sometimes this might appear to produce a promising result, yet the costs to the plant may be high and other responses and traits are affected.

It is of particular interest and concern that many of the scientific papers exploring the possibilities and reporting on genetic engineering experiments for particular stress tolerance traits, do not look at the potential or actual results of conventional breeding. They also do not look at the potential for altering external factors to reduce the stress on plants.

### 5.2 Nutrition-related Traits

Rice is a long-established reliable major staple food,<sup>20</sup> with many nutritional qualities and a rich variety of flavours and properties. In combination with other foods such as pulses and greens, it is the foundation for a perfectly balanced diet.

There is a tendency, especially in some western food cultures, to reduce food variety and diversity and to "fortify" foods with minerals and vitamins in an effort to counterbalance such reduced nutritional value of the diet.

<sup>19</sup> EPSPS stands for 5-Enol-pyruvylshikimate-3-phosphate synthase. It is a key enzyme in the shikimate biosynthetic pathway and thus essential for the plant's production of aromatic amino acids, phytoalexins, folic acid, lignin and many other compounds. Whilst the plant's own EPSPS is inhibited by glyphosate, the EPSPS enzyme from Agrobacterium sp. CP4 is not. It is this CP4 EPSPS gene that is used in GE plants to achieve glyphosate tolerance.

<sup>&</sup>lt;sup>20</sup> A staple food source - such as rice, maize, wheat, potato or cassava - is never intended to be used as a sole food source but rather as the major energy source, i.e., starch.

Whilst the value and benefits of a varied and balanced diet cannot be replaced by nutrient "fortification" or "enrichment" of individual foods, this approach is nevertheless promoted and pursued by food processors and producers of "enhanced" foods.

In line with this approach, any lack of nutrients in a diet will result in a call for food enhancement rather than in efforts to increase food variety and diversity in the diet or to address underlying causes, such as poverty, lack of education or loss of cultural knowledge.

In this context, it has to be emphasized that GE rice engineered with nutrition-related traits will carry all the same health and environmental risks and problems as other genetically engineered organisms (see section on risks). Furthermore, a techno-fix cannot address social or cultural problems. Indeed, a technofix will usually worsen a situation in the medium to long run and take resources away from real solutions.

There are many readily available and safe food sources of nutrients such as vitamins, flavonoids, iron, zinc, special amino acids or oils. The motivation behind genetically engineering rice to contain such nutrients is therefore questionable, and seems, at best, misguided.

### 5.2.1 Pro-Vitamin A

First announced in 2000, Golden Rice is genetically engineered to produce beta-carotene and deliver Pro-Vitamin A as a remedy to wide-spread Vitamin A deficiency. An upgraded version, Golden Rice 2 [32], has been produced by Syngenta Corporation and is in the process of being field tested in Asia. Whilst the first version had extremely low levels of Pro-Vitamin A, Golden Rice 2 is said to quickly lose its Pro-Vitamin A level during storage. (See section on corporate involvement.)

Vitamin A deficiency is generally

viewed as a social problem requiring political answers, including better sanitation to reduce diarrhoea in children, as this prevents the absorption of vital nutrients, such as Pro-Vitamin A. Growing greens and vegetables in community plots or against house walls are projects supported by the WHO, FAO and many NGOs to enhance health and reduce Vitamin A deficiency. It is well known that Vitamin A deficiency is only one aspect of malnutrition and an indicator of a serious lack of a whole range of vital micronutrients that would be available in a balanced diet.

Golden Rice is clearly a technofix approach, incapable of solving the problems at hand, but rather taking resources and attention away from real solutions.

# 5.2.2 Flavonoids (or polyphenols)

These are plant compounds that give berries, fruits, vegetables or flowers their bluish-red or yellow colour, many flavonoids are thus referred to as "plant pigments". Rich sources of flavonoids are also green and black tea, red wine, garlic and onions. Flavonoids have antioxidant, anti-inflammatory and immune-supporting properties. Consequently, consuming vegetables and fruit, or even drinking tea, are highly beneficial to one's health.

Given that flavonoids are widely available in various vegetables, fruits and leaves, it is hard to understand why biotechnologists would want to make white rice plant cultivars produce flavonoids. Yet this is exactly what Korean scientists were attempting to do when genetically engineering white rice cultivars with maize genes coding for flavonoids, resulting in low expression. [33] Such research is a clear example of misguided development and a waste of resources.

#### 5.2.3 Iron and Zinc

In order to make rice a source of iron, rice has been genetically modified with human lactoferrin, normally found in human breast milk. Field trials have been carried out in the US and Ventria Bioscience is-according to its own website and patent claims—aiming to market this rice as a supplement in infant formula. Whilst this may attract or satisfy investors or some media, the documents submitted to the USDA and FDA for approval in the US do not mention infant formula, which requires strict testing. Rather, the request is for approval as a sports energy drink, a food supplement. Others have used the ferritin gene from soybean to increase iron and zinc levels (e.g., [34]).

As iron and zinc are readily available in other food sources, such as beans and whole grains, it is again unnecessary and questionable to add new risks and hazards to a staple food source through genetic engineering.

#### 5.2.4 Amino Acids

Amino acids are the building blocks of proteins. Found in high levels in animal products, other common sources of amino acids are beans and pulses which are traditionally eaten in combination with rice. In an attempt to turn rice into a valuable protein source, it has been genetically engineered to produce elevated levels of tryptophan (e.g., [35, 36]), glycins, cysteine, and methionine. Tryptophan-rich rice has been the subject of field trials in Japan.

However, good plant sources of tryptophan already available are oats, bananas, chocolate, dried dates, sesame, chickpeas, sunflower seeds, pumpkin seeds, spirulina and peanuts. It is also found in turkey at a level typical of poultry in general.

### 5.2.5 Oil Quality

Rice bran consists of approximately 20% oil. To alter the nutritional quality of rice bran oil, rice has been transformed with the omega-3 fatty acid desaturase gene from soybean. Experiments showed a clear increase of the oil alpha-linolenic acid (ALA). [37]

Other than fish, seafood and meats, common sources of omega-3 fatty acids are seed oils for example, from flax (linseed), chia sage, kiwifruit and perilla. Butternuts and walnuts are another good source, so are brown algae (kelp) and acai palm fruit. Alpha-linolenic acid (ALA) is one of the most common forms of omega-3 fatty acids and can be found in all of the above and in many common vegetable oils including oil from soya, hemp, rapeseed (canola), and sea buckthorn.

There is no need to turn rice bran oil into an ALA source, especially when this requires genetic engineering which in itself has disruptive and unpredictable impacts and consequences, which in this case have not been investigated.

#### 5.2.6 Starch

To change the processing quality of rice, a number of groups are attempting to alter its starch composition, especially the amylose and amylopectin content. Both these molecules play a decisive role in the processing properties of rice. To facilitate the brewing of sake, GE rice was produced with low gluterin. Field trials were conducted in Japan in 2000 by the Orinova Company, reportedly a joint venture between

Japan Tobacco and Aventis, but this initiative was subsequently abandoned due to lack of consumer support.

## 5.3 PharmaCrops

There are numerous groups working on using rice as a bio-factory for pharmaceuticals. Best known in this are Ventria Biosciences, US, who are already growing GE Pharma-rice in limited conditions to produce pharmaceutical substances for marketing. Rice has been modified with human genes to produce lyzozyme, lactoferrin and the human serum albumin. Although not presently used for human consumption, Ventria is marketing their transgenic rice products through InVitria as an ingredient for cell culture medium used by labs, pharmaceutical companies and hospitals. The website states for example: "Lacromin™, recombinant human lactoferrin, is a plant-derived recombinant growth factor for cell culture media that improves productivity and safety. Lacromin™ is a strong growth factor and outperforms Transferrin, a common animal-derived growth factor."21 Feeding trials with Ventria's lactoferrin rice on infants with diarroheoa in a Peruvian hospital in 2006 were reported in the press and attracted wide criticism.<sup>22</sup>

Other research groups also report on rice transformed with human lactoferrin and lysozyme, as well as with human ovokinin — to be used to treat hypertension [38], and other illnesses. Rice has also been genetically engineered with pollen allergens, stated to have a potential application for allergy sufferers. [39]

#### 5.4 Industrial Traits

Some also envision the genetic engineering of rice to produce compounds for use in industrial processes, for example, in the production of industrial enzymes, such as transglutaminase, (used in the food processing industry as a binding agent to improve the texture of protein-rich foods such as surimi or ham) reported by a Spanish-German group in 2004. [40]

#### 5.5 Bio-remediation

The idea of using transgenic plants to clean up land which has been contaminated with toxic pollutants by industrial or agricultural processes has been around for a while. Rice genetically engineered with human cytochrome P450 genes was found to be tolerant to and to metabolise (degrade) various herbicides, such as atrazine and metolachloris. Japanese biotechnologists suggest that this rice should "prove useful in implementing effective, inexpensive methods of decreasing various chemicals that are widespread in agricultural environments." [41] They also state: "Assuming that public and commercial acceptance is forthcoming, pIKBACH rice plants may become useful tools for the breeding of herbicide-tolerant crops and for phytoremediation of environmental pollution by organic chemicals." [41] If such rice were to enter the food chain, this would raise huge ethical concerns for many people, who may mind consuming human gene variants. Furthermore, rice used for remediation of toxins will contain those toxins or traces thereof and thus should not be used for human or animal consumption. The accidental mixing of bio-remediation rice with ordinary rice during any stage of growing, transportation and processing has thus to be strictly avoided. All of the present GE commodity crop production chains have, however, been unable to keep GE crops strictly separate from their conventional counterparts.

<sup>21</sup> InVitria website: http://www.invitria.com/ products/lacromin.html

For example: U.S. Laboratory Testing Transgenic Rice On Peruvian Children. 16 June 2006. Eduardo Aragon from TeleSur in Venezuela. Translation posted on Free Internet Press - http://freeinternetpress.com/ story.php?sid=7256

# 6.0 Who and What Is Driving GE Rice?

The actors, interests and motivations behind the drive for GE rice are similar to those of the Green Revolution, with a few new additions, such as pharmaceutical and bio-fuel production. As during the Green Revolution, concern is being expressed as to how to feed growing populations. Everyone involved is pointing at the predicted population figures of 8.9 billion by 2050 while some refer specifically to the numbers suffering from hunger and malnutrition today. For both scenarios, the main solution being put forward is technology and the scientific community is primed to deliver this solution via genetic engineering. This is articulated by T. Sakamoto from the University of Tokyo: "Recent advances in plant genome analyses and plant biotechnology will realize a second Green Revolution through the genetic engineering of food crops." [42]

The Rockefeller Foundation focused attention on rice in the mideighties when it initiated its "International Rice Biotechnology Program". During the 15 years (1984-1999) that followed, the Foundation spent USD105 million on furthering skills and knowledge in the field of rice biotechnology and the development of GE rice varieties. Funding went to the North as well as to the South. The development of Golden Rice, for example, was a project supported by the Foundation. So were projects focussed on genetically engineering rice for traits requiring only single genes, for

example, pest and disease resistance. Initial results were often presented as great successes and proof that GE rice was the way towards food security.<sup>23</sup> The Rockefeller Foundation was intent on including rice in the Gene Revolution. [44] In the meantime, rice had become a major model plant for cereal research and development.

# 6.1 The Race for the Rice Genome

The project to obtain the complete DNA sequence of all the genetic information (genome)<sup>24</sup> of rice was started by Japan in the 1990s. Eventually, in 1997, the International Rice Genome Sequencing Project (IRGSP) was launched as a public project — using the Nipponbare or GA3 variety from Japan.<sup>25</sup>

It was widely recognised that having genetic information on rice also opened up a gateway for all other cereal food crops. Furthermore, the reward was not only knowledge; patent protection could also be secured. For these reasons, private companies quietly joined in the race.

In April 2000, the agro-biotechnology company Monsanto announced it had completed its own draft sequence for the rice genome, which still had numerous gaps. Though offering to share the information, control over the intellectual property remained largely with Monsanto.

In January 2001, another draft version was announced by the agrobiotechnology company, Syngenta.

In December 2002, the IRGSP finally announced the completion of a high quality draft sequence, which was finalised and published by August 2005.

In the meantime, Chinese scientists had worked on the sequence of *Indica* rice – the main rice variety in China and many Asian countries. In April 2002, their draft sequence was published in *Science*. [45]

## 6.2 Company Involvement

Transnational biotechnology companies that have clearly stated their interest in marketing/commercialising GE rice are Monsanto, Bayer, Syngenta and the seed company Delta & Pine Land.

- a) Monsanto, a US-based agrochemical corporation and world number one seed company, has developed both herbicide-tolerant as well as insect-resistant genetically engineered rice. Their Roundup Ready rice (RR rice) is tolerant to Monsanto's glyphosate-based herbicide Roundup. Since 1998, about 50 field trials have been carried out in the US. In India, Monsanto is also known as Mahyco-Monsanto Biotech (MMB) or in the form of its seed partner, Mahyco (Maharashtra Hybrids Seeds Co Ltd). Having already commercialised its Bt cotton (Bollgard) in India, Monsanto wants to commercialise its Bt rice first in India. Field trials are presently taking place in a number of states, provoking widespread objection. Another food crop genetically engineered for insect resistance using Bt toxins is Bt okra (lady's fingers), which is also undergoing field trials.
- b) Bayer CropScience, with headquarters in Germany, took over from Aventis CropScience, which used to be AgrEvo. It is a company haunted by GE contamination scandals. The first was 'Starlink', a genetically modified Bt maize not approved for human consumption but grown as animal feed. This maize found its way into the human food chain on a global scale in 2000 and 2001, including in European Union countries and Japan. Products had to be withdrawn from the shelves internationally.

<sup>&</sup>lt;sup>23</sup> e.g., http://www.news.cornell.edu/releases/ June96/rice.hrs.html (see also endnotes [25, 43, 44])

<sup>&</sup>lt;sup>24</sup> Genome = complete set of genetic information of an organism.

<sup>25</sup> http://www.genoscope.cns.fr/externe/English/ Projets/Projet\_CC/organisme\_CC.html

In 2006, one of Bayer's genetically engineered herbicide-resistant rice strains (LL601) contaminated rice crops in the USA, severely affecting its international export market. Though only ever grown in trial plots, it managed to contaminate two top rice varieties grown in the US, namely Cheniere and Clearfield 131. It actually contaminated rice supplies to Europe and other countries. (See section on herbicide tolerance for details).

c) Syngenta is involved with Golden Rice<sup>26</sup>, especially Golden Rice 2 (GR2). In 2000, Syngenta (then Zeneca) emerged as the main actor with regard to patents and licensing agreements for the original Golden Rice (GR1). Part of the agreement reached was that "resource-poor farmers in developing countries" would not have to pay royalties or technology fees as long as they generated less than USD10,000 annually.<sup>27</sup>

The original Golden Rice was mostly designed and created in public laboratories. The levels of beta-carotene (Pro-Vitamin A) produced by this Golden Rice were very small and widely criticised. Golden Rice number 2 (GR2) is based on the original design, but uses fewer or different genes and is said to produce higher levels of Pro-Vitamin A than its predecessor. GR2 was solely developed by Syngenta,

Experience and data gathered with GR2 so far seem to indicate that the beta-carotene levels achieved in the harvested grain drop quickly during storage, thus not offering the expected improvement. Given the true causes of Vitamin A deficiency (VAD), GR2 offers no more effective an answer to VAD and malnutrition than the original Golden Rice. The following commentary from the book Hungry Corporations therefore still holds true: "The problem is not a lack of foods containing Vitamin A and beta-carotene, but a lack of access to these foods. It is 'hidden hunger', including the loss of knowledge about the relation between diet and health, and the consequences of eating only rice. Furthermore, Vitamin A and beta-carotene are fat-soluble nutrients and can only be properly absorbed in the presence of oil and other components. Children who suffer from diarrhoea due to dirty water and poor hygiene conditions will not be able to take up or retain nutrients like Vitamin A from their food." [1]

Many plants are sources of Pro-Vitamin A, especially carrots, yellow cassava, yellow sweet potato, mangoes and apricots (also in dried form), leafy greens such as spinach, coriander, radish leaves, and, most of all, red palm oil.

As mentioned above, Syngenta has itself sequenced the rice genome, created its own database and secured its own patents. It

has also developed GE rice varieties and conducted ten field trials in the US: six for herbicide-resistance, two for insect-resistance and two for seed composition.

d) Delta & Pine Land (DPL) is the world's largest cotton seed company. DPL bought Syngenta's global cotton seed business in May 2006, and has now, for example, cotton seed germplasm and distribution assets in each of the three primary cotton-growing regions of India. Monsanto announced in August 2006 that it was to buy DPL for USD 1.5 billion. Whilst there was opposition, the sale eventually went through.

DPL is not only the largest cotton seed company, but also the inventor and co-patent holder of "Terminator Technology". "Terminator" is a genetic engineering technology that makes plants grow sterile seeds. Farmers can thus no longer save seeds but need to buy their seeds from seed companies every year. There is an international campaign by civil society to ban terminator technology, and the UN Convention on Biological Diversity has declared a moratorium on it. DPL have repeatedly asserted that their aim is to develop the sterile seed technology for three plants: soya, wheat and rice.

The consequences of rice seeds being marketed with terminator technology is illustrated by the following statistics, supplied by the ETC in 2006: In the Philippines, 59% of the rice crop is planted with farmer-saved seeds. If these rice farmers were forced to buy new seed every time they planted - they would spend an estimated USD 172 million per annum.<sup>28</sup>

which used the attention of World Food Day on 16 October 2004 to announce the donation of its GR2 to the Golden Rice Humanitarian Board, under the same conditions and licensing terms as the previous Golden Rice.

<sup>26</sup> Golden Rice is GE rice genetically engineered to produce Pro-Vitamin A (beta carotene) in the rice grain, which will change the colour of the grain to a pale orange. First announced in 2000, this original version was engineered with five extra genes, mostly from daffodils.

<sup>27</sup> http://www.goldenrice.org/Content1-Who/ who\_Gary.html

<sup>&</sup>lt;sup>28</sup> ETC group document (March 2006): The Potential Economic Impact of Terminator Seed Technology - Estimates for Selected Crops and Countries.

e) Ventria Bioscience, with its headquarters in California, US, is aiming "to become a scientific leader in the biopharmaceutical industry." Their claim is that their science and patented technology "make it possible for Ventria to address unmet and underserved needs in human and animal health by delivering affordable treatments on a global scale." It is ironic that they should choose to use rice as their "production system" for pharmaceuticals, thus threatening to contaminate and undermine the world's most important food crop, upon which more than half the world's population depend for their daily needs.

In fact, there is a lot of money in pharmaceuticals, and plants appear to offer a cheap production system. Rice has been bred and selected by generations of farmers for thousands of years to efficiently produce proteins, starches and other compounds locked into a seed that can be stored easily until required for consumption. It is exactly this quality that is sought by pharmaceutical companies.

Others hold that this dream of rice or other plants being a "cheap production system" does not reflect reality but is ultimately rather expensive, and highly problematic with regard to contamination.

Ventria seems also to have chosen the cheaper option of marketing its GE rice and its GE rice proteins not as pharmaceuticals or infant formula, but as food and drink supplements. Thus it can

# **Dates Marking the Development of GE Rice**

	Dates marking the Development of GE Rice
1988	First transgenic rice produced (using direct gene transfer into protoplasts)
1991	Use of shotgun method or 'biolistics' for generation of rice transgenics
1993	Insect-resistance rice by using $\delta(\text{delta})$ -endotoxin ( <i>Bt</i> )  Transgenic rice with <i>bar</i> gene for sheath blight resistance
1994	First conclusive report of rice transformation by agrobacterium
1995	Resistance against bacterial blight obtained by using cloned Xa21 gene
1996	Insect-resistance transgenic Indica rice harbouring <i>Cry1Ab</i> gene Field trial of herbicide-resistant transgenic rice Insect-resistant transgenic rice harbouring <i>pin</i> II gene
1997	Use of reconstructed <i>Cry1Ac</i> gene for high-level resistance against pests
1998	Multigene transformation  First report of agrobacterium-mediated rice transformation for agronomically
	important genes ( <i>Cry1Ab and Cry1Ac</i> )  Terminator patent granted to Delta & Pine Land and USDA.
1999	Iron-fortified transgenic rice with soybean ferritin gene
1000	Resistance against rice yellow mottle virus (RYMV) derived by pathogen- derived resistance
2000	Field trial of hybrid rice containing Bt gene
	Golden Rice announced as the answer to Vitamin A deficiency  Monsanto announces its draft sequence of Japonica rice genome
2001	Syngenta announces its own draft sequence of Japonica rice genome
2002	Chinese researchers publish complete draft sequence of Indica rice Syngenta publishes Japonica draft sequence
	IRGSP's complete draft sequence of Japonica rice genome
2004	Completion of final Japonica rice genome sequence by IRGSP
2004	Golden Rice 2 presented by Syngenta
2006/7	The global Liberty Link (LL) rice contamination scandal
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Source: Based on Bajaj & Mohanty (2005) [28], with the author's amendments. Abbreviations: *Bt: Bacillus thuringiensis*; IRGSP: International Rice Genome Sequencing Program; *pin*II: potato proteinase inhibitor II gene; *Xa*: native rice gene for bacterial blast resistance; *Cry1Ab* and *Cry1Ac*: particular Bt endotoxin genes.

avoid the costs and the rigour of clinical trials. Nevertheless, it proposes it as an over-the-counter rehydration drink, especially intended for children in Third World countries suffering from diarrhoea.<sup>29</sup>

f) Other Players: It is beyond the scope of this paper to list all those involved in either genetically engineering rice, or facilitating, guiding and/or financing research and development on it. A quick look at the Golden Rice Humanitarian Board, for example, reveals some of the complexity: Next to scientists and inventors such as Prof. Ingo Potrykus and Peter Beyer, representatives of the following bodies are included: The International Rice Research Institute (IRRI), The Rockefeller Foundation, Syngenta, HarvestPlus, USAID, and the Department of Biotechnology (India). [46]

<sup>29</sup> North Sacramento-based Ventria sowing a storm with altered rice. By Jim Downing. Sacramento Bee, USA, 3. Nov. 2007. http:// www.sacbee.com/103/story/469124.html also kept at http://www.genet-info.org/

# 7.0 Risks and Implications Arising from GE Rice

Having discussed the problems of genetic engineering, followed by an overview of the risks, in Section 4, and having presented some of the main developments and players in the GE rice scenario in Sections 5 and 6 respectively, this section will look in more detail at the risks and implications arising from GE rice. These focus on health and food safety; biodiversity, environment and farming; and contamination and other risks.

# 7.1 Health and Food Safety

# 7.1.1 Special concerns

- a) Allergenicity: GE rice may produce a new allergen (a substance that evokes an allergic reaction) or may produce raised levels of allergens already present (e.g., particular glycoproteins in seed and pollen). This may vary depending on conditions and stresses the plants are exposed to. (See section on studies below.)
- b) Toxicity: A food component can be poisonous in itself or indirectly in combination with other substances. The latter is almost impossible to identify in tests. Both forms of toxicity may arise through GE. There is a further differentiation between acute and chronic low level toxicity. Acute toxicity is rapidly noticed, within minutes or at most hours after ingestion, and would most likely be picked up in well-

designed feeding trials. Chronic low-level toxicity occurs from ingesting food that is mildly toxic and will lead to harm and/or diminished health when eaten repeatedly over longer periods of time. Classic examples here are the effects of long-term exposure to pesticide residues. Such effects would be very difficult to pick up in feeding trials and would require long term monitoring. (See section on studies below.)

This raises serious concerns for any open-air field trial or any release where the GE rice in question has not been thoroughly tested for health implications. Given the recent contamination incidents of conventional rice by GE rice (LL601 & LL62)<sup>30</sup> that had only been used in field trials some three years previously, the status of "field trial" should be no excuse for lack of thorough and extensive safety and risk assessments.

# c) Further concerns are:

- changed level or composition of anti-nutrients.
- changed nutrient content and composition,
- · nutritional incompatibilities,
- · synergistic effects.
- reduction of essential micronutrients, and
- gut bacteria acquiring antibiotic resistance due to horizontal gene transfer of such marker genes

# 7.1.2 Studies on health and safety risks

All these concerns require further discussion and research. There have been reports and scientific studies that give clear warnings. Whilst these warrant a separate paper, a few studies are briefly outlined below:

# a) The use of Bt toxins in rice is problematic.

Numerous studies have raised serious questions about the

potential health impacts of Bt toxins (such as the  $\delta$ (delta)endotoxins Cry1Ac or Cry1Ab). As detailed earlier, Bt rice in China is largely engineered with Cry1Ab, and Monsanto's Bt rice grown in field trials uses Cry1Ac. Work in the U.S. involving farmworkers exposed to Bt sprays found that 2 out of 123 had antibodies to the  $\delta$ -endotoxins Crv1Ab/Crv1Ac, [47] Dr. Steven Gendel of the US Food and Drug Administration found that Cry1Ab and Cry1Ac have significant sequence similarity to vitellogenin, a known egg allergen, and concluded that "the similarity between Cry1Ab and vitellogenin might be sufficient to warrant additional evaluation". [48, p.60] A series of studies published by scientists from Cuba and Mexico found that Cry1Ac is a potent immunogen, e.g., it evokes an immune response, and does so as potently as the cholera sub-toxin. It binds to gut cells and is capable of causing changes in the permeability of the gut (e.g., [49-52]). In fact, toxicological tests would be necessary to "demonstrate the safety of Cry1A proteins for the mucosal tissue and for the immunological system of animals".[51] A study by Dutch scientists found sequence similarity31 between Cry1Ac and cedar pollen allergen. [53]

Finally, the risk of immune response via inhalation is larger than the response from ingestion as inhaled substances are not exposed to gut digestive enzymes because they go directly into the circulatory system. In addition, some of the inhaled proteins can reach the digestive system via the connection between the nasal passage and the oesophagus.

News Release. Update for rice industry regarding Clearfield 131 long-grain rice seed – March 9, 2007. APHIS, United States Department of Agriculture. http:// www.aphis.usda.gov/newsroom/content/ 2007/03/CL131update3-9-07.shtml

<sup>31 (</sup>utilizing the methodology for sequence similarity recommended by the FAO/WHO 2001 Expert Consultation)

Implications of all the above studies still have to be researched. Depending on the type or strength of the immune response, allergic reactions can include anything from mild rashes or sneezing to severe and even life-threatening reactions, such as anaphylactic shock. Symptoms for example, experienced by farmers in India working with Bt cotton included skin. eye and respiratory ailments such as itching and sneezing.32 Delayed allergies or sensitivities can cause chronic conditions such as eczema, fatigue and migraines. Given the seriousness of existing allergies to food and pollen and the wide dependency on rice as a staple food, all risks should be avoided that could undermine the safe consumption of rice as a staple food.

## b) Examples of results of feeding trials: gut damage, and immune and allergic reactions

Already in the 1990s, in feeding trials with rats (and mice), GE tomatoes in the US (Clagene) as well as GE potatoes in the UK [54] [55] were found to cause damage to the gut and its mucosal cell lining. In both cases, the transgenes used were coding for proteins regarded as harmless. The questions raised by those trials have still not been answered. Furthermore, the tomato referred to was Calgene's FlavrSavr, which was actually sold on the US market between 1994 and 1996. The US Food and Drug Administration (FDA) had consciously ignored these findings in their assessments, as

documents later revealed in public hearings. [1]

Another study from 2005 looked at GE peas that had been genetically engineered with a bean gene. Unexpectedly, the protein product from the bean gene changed its characteristics and became immunogenic, i.e., causing immune reactions. [56] Hogan and his team from Canberra University, Australia, transferred the alpha-Al gene (alpha-amylase inhibitor) from the common bean to peas, which led to a number of astonishing results. The transgenic pea caused immune reactions and inflammation in mice, not seen in the bean. They found that although the original gene and the modified transgene both coded for exactly the same protein, the pea produced a structurally different protein from the same information. This is a very significant finding, as it provides evidence that a gene may behave differently when transferred from one organism to another, even if the two organisms are very close from an evolutionary standpoint. Yet there was more to the study: When mice were fed the transgenic protein together with other proteins commonly found in seeds, they also developed an immune response to some of these proteins, such as pea globulins, lectin, and vicilin-4. This is referred to as "immunological cross priming", also known as an "adjuvant effect". In essence, the process of genetic engineering has created a situation where the immune system will react to proteins that it has previously ignored, i.e., proteins that normally are not immunogenic, become immunogenic. Such increased stimulation of the immune system is not beneficial but in fact can be very dangerous. Asthma, allergies and hay-fever are some common symptoms of an overactive immune system.

The consequences of such a scenario for GE rice would be that even if it were possible to recall all GE rice and stop growing it, people would have started to develop allergies to other substances, present in ordinary rice or other foods. This could not be remedied by any recall or withdrawal of GE rice.

Feeding trials conducted or evaluated by other research groups found GE food crops triggering immune responses, affecting development, size and function of key body organs, as well as of red blood cells (e.g., [57, 58]).

# 7.2 Biodiversity and Environment; Agrobiodiversity and Farming

The potential implications of GE rice for the living environment as well as for water, soil and agricultural practices are serious.

Sustainable agriculture, for example, depends on a functional and supportive soil food web. It depends on a surrounding biological system that can act as a support to crops and a buffer in times of extreme conditions. Furthermore, it depends on diversity, including agro-biodiversity with good quality seeds.

#### 7.2.1 Soil

"A key factor for crop health is a functional soil food web, as this determines the fertility of the soil and its capacity to break down organic and inorganic substances such as herbicides, as well as to drain or hold water. The soil food web is a complex, interactive and interdependent system of mutually beneficial

<sup>&</sup>lt;sup>32</sup> Impact of Bt cotton on farmers' health (in Barwani and Dhar District of Madhya Pradesh) – Dr. Ashish Gupta et al. Investigation Report Oct. – Dec. 2005 www.gmwatch.org/printarchive2.asp?arcid=6265

soil organisms made up of microorganisms such as bacteria, fungi, algae and protozoa as well as insects, nematodes and earthworms. A teaspoonful (or one gram) of soil can contain 1–600 million micro-organisms from 5–25,000 different species, with conventional agricultural soil often lying in the lower range. In addition, soil is also made up of minerals, nutrients, air pockets, roots and decaying matter." [11]

In fact, soil organisms are not only vital to plant health, but also to soil health, structure and water-retaining properties, nutrient cycling, and the accessibility and transport of nutrients to plants. In exchange, plants excrete nutrients such as sugars from their roots as extra food for the micro-organisms. *Mycorrhizae* (or root fungi), for example, are particularly important because they link plant roots with the soil and play a vital role in the plant's uptake of nutrients.

# Implications for soil from GE rice

There are five main scenarios to consider with regard to the risks to soil from GE rice:

- a) the transgenic compounds, such as Bt toxins, exuding from the roots into the soil:
- b) the impact of repeated applications of the same broad-spectrum herbicide on soil;
- c) altered decomposition time of plant material;
- d) altered or disrupted plant-microorganism interaction; and
- e) horizontal gene transfer from GE rice to soil organisms.

Points (a) to (d) are briefly discussed below.

# a) Releasing of transgenic compounds

Plants exude substances into soil for various reasons, for example to enable food uptake; for defence against or attraction of microorganisms; or providing food to beneficial microorganisms. Although one study undertaken in China reported that no Bt residues (Cry1Ab) could be measured in the root-sphere of a particular Bt rice (KMD) [59]. the release of Bt toxins into the soil from genetically engineered Bt corn has been observed on a number of occasions (e.g., [60, 61]). Indeed, it is not understood when, or under which circumstances and conditions a Bt plant will leach or exude Bt toxins into the soil or whether such leaching may even be "event specific" (specific for this particular GE crop line).

The implications are not yet understood as it is still unclear for what purpose the Bt toxins (here. delta-endotoxins) are being produced by Bacillus thuringiensis, the soil bacterium used as the gene donor in the creation of Bt crops. In fact, within the bacterium, the endotoxins are present as so-called "pro-toxins" which are kept in the form of crystalline bodies (inclusions). If such crystalline pro-toxins are ingested by insect larvae,33 they will become soluble in the gut of the larvae if the acidity level (pH) is right. The pro-toxin will only become an active toxin once a section has been removed by a digestive enzyme. In this form it can attach to receptors in the gut wall, which it will then perforate. Many of the transgenic Bt toxins have been altered so that unlike the original pro-toxins, they are already partially activated in the GE plant (as sequences in the front of the gene

have been removed). Whilst little is known of the impact of the original bacterial Bt pro-toxin on soil organisms—whether these are micro-organisms, soil dwelling insects, spiders, or earthworms—even less is known of the impact of the genetically engineered protoxins produced by Bt plants.

As long as the impacts on soil, soil organisms, nutrient cycling and plant-microorganism signaling are not understood, it is ill advised to grow Bt plants, as it might for example, contribute to weakened plants and poor harvests. Different studies with Bt toxins (Cry1Ab) have for instance, shown ill effects, such as weight loss [62] and cocoon hatchability [63], on earthworms.

Leaching or release of other GE compounds and the impacts on soil and the soil food web will need to be considered as other GE rice applications are being tested and developed, such as GE Pharma-rice.

b) Repeated applications of broad-spectrum herbicides when growing herbicide-tolerant GE rice (e.g., glufosinate for LL Rice and glyphosate for RR rice)

There are studies for both these herbicide regimes that illustrate their effects and impacts on the composition of the soil micro-organism community. Both have, for example, been reported to lead to increased levels of pathogenic fungi—such as fusarium in the case of glyphosate—as shown for herbicide-tolerant GE soybean [64] and GE wheat [65].

### Altered decomposition time for plant matter observed in GE plants

Whilst plant material from Bt maize has been found to decompose more slowly than conventional maize, the opposite effect

<sup>33</sup> In certain types of agriculture, including organic farming, fields infested with leaf eating insect larvae are sprayed with solutions of *Bacillus thuringiensis* or Bt protoxin preparations. Pests feeding on the leaves will thus ingest the pro-toxin.

was reported for a Bt cotton experiment. American researchers observed increased microbial activity for 2 out of 3 GE Bt cotton lines, when leaves had been placed in soil. Although these are Bt plants, the effects are thought to be due to GE transformation effects rather than to the transgene itself. [16] This illustrates that the quality and extent of plant-soil organism interaction cannot be prejudged on the basis of the introduced trait alone, but needs to be tested for each and every transformation event. On the whole, Bt-induced ecological shifts in the microbial communities of cropland soils are being observed, e.g., the addition of Bt maize residue leading to a rapid change in bacterial activity and composition as compared to non-Bt maize residue. [66, 67]

# d) Altered or disrupted plantmicroorganism interaction

GE crops have also been found to have a reduced interaction with beneficial soil organisms. Italian researchers found for example, that the roots of Bt maize lines (containing a *Cry1Ab* gene) had significantly less colonization by beneficial and symbiotic root fungi (mycorrhiza, e.g., *Glomus mosseae*) and reduced levels of beneficial soil bacterial association. [67]

### 7.2.2 Insects and Arthropods

There has been a lot of discussion on how best to assess the impact of GE traits on arthropods, especially on beneficial predators or pollinators. Central to the discussion and investigations are crops genetically engineered for insect resistance, whether this is with Bt toxins, lectins or proteinase inhibitors. Herbicidetolerance is also a trait under investigation, as herbicides can impact in-

sects or spiders both directly and indirectly. A big problem though, is that there is still no agreement on what would be the right method of experimentation, which arthropods should be tested and indeed how to interpret the results (e.g., [68, 69]). For example, Swiss researchers were able to show that predators of plantfeeding insects (pests) can die from Bt toxin when feeding on larvae that had previously ingested Bt crops. The predator Chrysoperla carnea (lacewing) died preying on the Bt crop-fed European corn borer. [70] Though a solid piece of experimentation with clear results, there is a sense of denial amongst transgenic Bt crop proponents who try to find fault with or disprove this, rather than using the precautionary approach. [71]

There is, however, a growing understanding that insecticidal GE crops such as Bt rice should not be allowed to negatively impact on nontarget organisms, especially beneficial ones. There is more agreement that effective measures should be taken to prevent target insect pests developing resistance to Bt toxins, e.g., by having refugia (where no Bt crops are grown) next to Bt crops, where pests can take refuge for feeding and breeding so that they do not rapidly evolve resistance through being constantly exposed to the toxin. Nevertheless, it is widely understood that pests will inevitably overcome GE resistance traits over time, necessitating either more genetic engineering and pesticide use, or a rethink of agricultural systems and practices.

One crucial point rarely discussed is that of the **emergence of new pests**. There have been a number of reports that Bt crops are coming under attack from insects that never previously behaved as pests towards the non-GE versions of the same crop.<sup>34</sup> This suggests that a

new ecological niche is being created by Bt crops, a niche for insects that are not affected by the Bt toxin and that are able to live and feed on the Bt crop. Whereas they could not previously compete against the pests targeted by Bt, an opening is being created by the elimination of these pests. It has also been found that some insects may prefer GE varieties to the non-GE versions, and thus become pests (see previous footnote).

# 7.3 Contamination and Other Risks

There are many more issues that need to be considered when looking at the risks to the environment, sustainable farming and biodiversity. The issue of weeds, for example; when using a herbicide-tolerant crop, any plant other than the crop plant is automatically defined and treated as a weed, despite the fact that such plants may well be important to sustainable agriculture and biodiversity conservation. There is also the potential of GE rice itself turning into a weed, or cross-pollinating with related weeds, giving rise to new pest-resistant and herbicide-tolerant weeds. Furthermore what will happen to neighbouring non-GE rice, i.e., the conventional and local rice varieties? Though highly self-pollinating (selfing up to

 $<sup>^{\</sup>bf 34}$  For example: Wang S, Just D R and Pinstrup-Andersen P. (2006). Damage from Secondary Pests and the Need for Refuge in China. pp. 625-637 In Regulating Agricultural Biotechnology: Economics and Policy. Richard E. Just, Julian M. Alston and David Zilberman (Eds.). 2006. Volume 30 in book series "Natural Resource Management and Policy". Springer US. A conference presentation by Wang et al. can be downloaded at: www.grain.org/ research\_files/SWang\_tarnished.pdf. Unfortunately the authors have made an erroneous conclusion, suggesting that a Bt refuge regime would get rid of the secondary pests. They wrongly assume that the target pest and the secondary pest are competitors. In reality, these pests feed on different components of the plant; one chews tissue, the other sucks juices.

99%), rice can and does out-cross, i.e., will pollinate neighbouring crops. Therefore, there is a real likelihood of GE rice lines contaminating conventional rice varieties—such a process is irreversible, holding the potential for the permanent loss of precious local varieties.

Whilst rice is well adapted to grow under flooded conditions for extended periods of time, the vast maiority of plants are not. Planting rice in flooded conditions stops weeds from growing and compete for light, nutrients and space. If rice is grown under (mostly) dry conditions, other plants will grow. This is where biotech corporations like Monsanto and Bayer believe their herbicide-tolerant rice should be grown, as weeds would not need to be manually removed but would be killed by herbicide applications. Yet what would be the effect of the herbicide drift to the neighbouring fields when spraying the GE field? Research, for example, shows that glyphosate drift on rice leads to severe yield loss. [72]

Widespread contamination of conventional rice by GE rice has already taken place, sources being LL rice in the US and Bt rice in China. Neither of these has been grown on a commercial scale, which suggests that contamination is inevitable even from small sources, including field trials. The mechanisms by which these large scale contaminations took place are not yet clear in either case but the implications are nevertheless daunting.

Some are proposing to use Terminator Technology to make rice produce sterile seed in order to prevent contamination in the field. There are two major flaws in this approach. Firstly, Terminator plants are not being designed to create sterile pollen, only sterile seed. Thus pollen from GE Terminator rice could

still contaminate neighbouring rice fields. Secondly, Terminator technology is a highly complex and unreliable design, with no prospect of ever working to the requirements necessary for biosafety; in fact, Terminator technology is more of a concept than a physical reality. Yet if we were to suppose for the purposes of argument that Terminator did exist, its impact would be to take away farmers' rights and ability to save seeds and improve their own varieties. It would very effectively do what it was originally designed to do: create a complete dependence on seeds sold by seed companies. Terminator is not the answer to biosafety concerns as it cannot deliver 100% reliability and effectiveness. [73]

Indeed, the only way to prevent contamination of food supplies, traditional rice varieties, seed supplies, and wild/weed relatives is by not growing GE rice.

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