Transgene Escape from GM Crops and Potential Biosafety Consequences: An Environmental Perspective

By Bao-Rong Lu

The Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, Fudan University, Shanghai 200433, China. E-mail: brlu@fudan.edu.cn

Abstract

The rapid progress of transgenic biotechnology has significantly promoted the development and production of genetically modified (GM) crops. The extensive global cultivation of GM crops has generated great benefits, which may provide opportunities for solving the problems inherent in world food security, but it has also aroused considerable biosafety concerns worldwide. Among these, the potential environmental consequences created by possible transgene escape from a GM crop to its non-GM crop counterparts (crop-to-crop) and wild or weedy relatives (crop-to-wild) via gene flow are the most debated biosafety issues internationally. Gene flow indicates the movement of genes or genetic materials from one plant population into another. There are three avenues for gene flow to occur: pollen-mediated, seed-mediated, and vegetative-propagule-mediated gene flow. There are a range of predicted possible environmental consequences (e.g., creation of new weeds, change of fitness-related characters, and loss of genetic diversity in crop landraces and wild relatives) caused by crop-to-crop and crop-to-wild transgene flow. In addition, transgene flow also arouses biosafety concerns for food/feed and health (e.g., GMO "contamination"), and socio-economics and ethics (legal and trading difficulties). Through pollen-mediated gene flow, a transgene can move from a GM crop into populations of a wild relative, and persist or disseminate in the wild population through further hybridisation and introgression between the GM crop and wild relative. If a transgene can express in wild plants as it does in the GM crop, the transgene may change a certain trait (e.g., insect resistance and herbicide tolerance) of the wild plants, possibly leading to further undesired consequences. If a transgene can alter the fitness of wild plants and the dynamics of the wild populations, the introgression of the transgene in the wild population may cause either local extinction of the population, by the so-called "swarm effect" (in the case of reducing fitness of wild plants), or make the wild population more invasive and competitive (in the case of increasing fitness of wild plants). A risk assessment system of the potential environmental consequences caused by transgene escape to wild relatives through

pollen-mediated gene flow can be developed based on the following science-based and step-by-step principles: 1) estimating the frequencies of transgene flow, 2) determining the expression level of the transgene in wild plants, and 3) measuring the fitness change brought about by the expression of the transgene in wild plants and populations. A systematic risk assessment will facilitate the appropriate prediction of potential environmental consequences caused by transgene escape to wild relatives under different circumstances. To minimise the possibility of transgene flow, a number of confinement strategies have been developed or proposed, applying physical and biological approaches. A transgenic mitigation (TM) strategy is also available for reducing the potential risks of escaped transgene(s) to the weedy or wild populations by co-introducing "mitigator" genes that are tandemly linked to the target transgene(s) to deliberately reduce the fitness of any hybrids and their progenies. The proper combination of transgene confinement and mitigation strategies will provide an effective management tool for minimising any environmental consequences created by transgene escape to wild relatives via pollen-mediated gene flow.

Riassunto

Il rapido progresso delle biotecnologie transgeniche ha promosso significativamente lo sviluppo e la produzione di colture geneticamente modificate (OGM). La coltivazione estensiva di OGM a livello globale ha generato grandi benefici, che possono fornire opportunità per risolvere i problemi inerenti la sicurezza degli alimenti a livello mondiale, ma ha anche generato preoccupazioni considerevoli in termini di biosicurezza. Tra queste preoccupazioni, le possibili conseguenze a livello ambientale create dalla possibilità di trasferimento genico da una coltura transgenica alla sua controparte non transgenica (da coltura a coltura) o a specie selvatiche e infestanti strettamente correlate (da coltura a specie selvatica) mediante flusso genico, sono l'argomento di biosicurezza più dibattuto a livello internazionale. Il flusso genico indica il movimento di geni o di materiale genico da una popolazione di piante ad un'altra. Ci sono tre vie attraverso le quali può avvenire il flusso genico: mediato dal polline, mediato dai semi, o mediato da propagazione vegetativa. C'è un numero di possibili conseguenze ambientali (ad esempio, creazione di nuove infestanti, cambio di caratteri legati all'adattamento, e perdita di diversità genetica in specie coltivate e specie selvatiche relative) causate dal flusso genico tra colture a da coltura a specie selvatica. Inoltre, il flusso genico determina anche preoccupazioni per la sicurezza degli alimenti e della salute (contaminazione da OGM) nonchè socio-economiche ed etiche (difficoltà legali e commerciali). Attraverso il flusso genico mediato dal polline, un transgene può spostarsi da una coltura transgenica a popolazioni di specie selvatiche geneticamente correlate, e persistere o diffondere nella popolazione selvatica mediante ulteriore ibridazione e introgressione tra la coltura transgenica e la selvatica parente. Se un transgene può esprimersi in una pianta selvatica come nella pianta transgenica, il transgene può cambiare una certa caratteristica (ad esempio, resistenza agli insetti e tolleranza agli erbicidi) della pianta selvatica, portando così ad ulteriori conseguenze indesiderate. Se un transgene può alterare le capacità di adattamento di una pianta selvatica e la dinamica della popolazione, l'introgressione del transgene nella popolazione selvatica può causare o un'estinzione locale della popolazione, mediante il cosiddetto "effetto sciame" (nei casi di ridotta capacità di adattamento delle piante selvatiche), oppure rendere la popolazione selvatica più invasiva e competitiva (nel caso di un aumento della capacità di adattamento delle piante selvatiche). Un sistema di valutazione del rischio di possibili

conseguenze ambientali causate da una fuga di un transgene a una specie selvatica relativa attraverso flusso genico mediato da polline può essere sviluppato sulla base dei seguenti principi, sia scientifici che passo dopo passo: 1) stimando le frequenze del flusso del transgene, 2) determinando il livello di espressione del trangene nella pianta selvatica, 3) misurando il cambiamento della capacità di adattamento determinato dall'espressione del transgene nelle piante selvatiche e nelle popolazioni. Una valutazione del rischio sistematica faciliterà una previsione appropriata delle conseguenze ambientali possibili causate dalla fuga del transgene nelle piante selvatiche in differenti circostanze. Per minimizzare la possibilità di un flusso del transgene, sono state sviluppate e proposte diverse strategie di confinamento, applicando approcci sia fisici che biologici. È anche disponibile una strategia di mitigazione transgenica per ridurre i rischi potenziali di fuga dei transgeni nelle popolazioni infestanti o selvatiche mediante co-introduzione di geni mitigatori che sono legati in tandem al transgene bersaglio per ridurre deliberatamente l'adattabilità di qualsiasi ibrido e della progenie. Una appropriata combinazione di strategie di confinamento e mitigazione del transgene fornirà un efficace strumento di gestione per minimizzare qualsiasi conseguenza ambientale creata dalla fuga del transgene verso piante selvatiche relative attraverso flusso genico mediato da polline.

1. INTRODUCTION

The rapid development of biological science and technology has brought the world into a new era of biotechnology (Lu, 2004; Huang *et al.*, 2005; Hatti-Kaul *et al.*, 2007). One of the most important characteristics of the biotechnology era is the wide application of genetic engineering (also referred to as genetic modification) technologies for the improvement of plant, animal, and microorganism species for human benefits. As a consequence, modern biotechnology has greatly promoted the research and development of genetically modified (GM), or transgenic crops worldwide. To date, a large number of modified genes conferring diverse traits have been successfully transferred into crop varieties through modern biotechnology (Christou, 1997; Hansen and Wright, 1999; Repellin *et al.*, 2001; Lu and Snow, 2005; Lee *et al.*, 2006; Zhao *et al.*, 2007). These traits include high protein content and unique nutritional compounds (Gura, 1999; Hasler, 2000; Ye *et al.*, 2000), disease and insect resistance (Datta *et al.*, 1998, 2002; Rao *et al.*, 1998; Huang *et al.*, 2005; Bock, 2007), virus resistance (Shepherd *et al.*, 2007; Vanderschuren *et al.*, 2007), herbicide resistance (GalloMeagher and Irvine, 1996; Lutz *et al.*, 2001; Toyama *et al.*, 2003), as well as salt and drought tolerances (Bahieldin *et al.*, 2005; Tang *et al.*, 2006). The great success of GM crops has had an enormous impact on world crop production and cultivation patterns of agricultural species such as cotton, soya bean, oilseed rape, and maize (James, 2006).

On the one hand, the commercial production of GM crops with various agronomically beneficial traits has opened a new dimension for meeting the great challenge of world food security by enhancing the efficiency of crop production. But on the other hand, the extensive environmental release and cultivation of GM crop varieties have aroused tremendous biosafety concerns and debates worldwide (Stewart *et al.*, 2000; Ellstand, 2001, 2003; Pretty, 2001). Biosafety issues have already become a crucial factor in constraining the further development of transgenic biotechnology and the wider application of GM products in agriculture. Nowadays, it is not possible to circumvent biosafety issues when discussing the development and application of GM crops in the world. Therefore, it is a rational attitude to face the challenge of those biosafety issues aroused by the cultivation of GM crops and try to close the "knowledge gap" by providing solid data from sound scientific research. Understanding what is the meaning of biosafety and its related issues will help make a correct decision when facing and dealing with the technology and its products. Biosafety refers to *"the avoidance of risk to human health and*

safety, and to the conservation of the environment, as a result of the use for research and commerce of infectious or genetically modified organisms" (FAO Glossary, http://www.fao.org/biotech/index_glossary.asp). Therefore, this terminology is related to the safety aspects of GM products from transgenic biotechnology that may pose an impact on human health and environment during the entire procedures of research, exploration, production, and utilisation. There are a quite number of biosafety-related concerns in general, but the most important ones can be summarised as follows:

- food, feed, and health safety caused by GM products (Aumaitre *et al.*, 2002; König *et al.*, 2004; Cromwell *et al.*, 2005; Hothorn and Oberdoerfer, 2006; Marshall, 2007);
- environmental safety (Dale *et al.*, 2002; Conner *et al.*, 2003; Celis *et al.*, 2004; Pilson and Prendeville, 2004; Sanvido *et al.*, 2007);
- labelling of GM products and the detection of possible transgene (or derived protein) presence in agricultural products (Ahmed, 2002; Matsuoka *et al.*, 2002; Phipps *et al.*, 2003; Vogel, 2006);
- socio-economical and ethic concerns aroused by the application of GM products and technology (Pray *et al.*, 2002; Finucane and Holup, 2005; Aerni, 2007; Einsele, 2007);
- regulatory procedures and acts for GM related issues (Schilter and Constable, 2002; Williams, 2002; Nap *et al.*, 2003; Novoselova *et al.*, 2007; Ramjoue, 2007; Spök, 2007);
- general public perception or acceptance of GM products and transgenic biotechnology (Yang *et al.*, 2005; Curtis and Moeltner, 2007; Horlick-Jones *et al.*, 2007; Huffman *et al.*, 2007; Knight *et al.*, 2007); and
- risk assessment systems in relation to the environmental release and cultivation of GM products (Ervin *et al.*, 2003; Andow and Zwahlen, 2006; Birch, *et al.* 2007; Hooftman, *et al.* 2007; Johnson *et al.*, 2007).

The concerns of environmental or ecological biosafety aroused by the introduction of GM organisms are the most challenging issue, because it is difficult to determine the long-term environmental impacts caused by GMOs released to the environment in which unpredictable and complex situations are always expected. However, the most discussed environmental biosafety issues can be summarised as follows:

- direct and indirect effects of toxic transgenes (e.g. the *Bacillus thuringiensis* [*Bt*] insect resistance gene) on non-target organisms,

and impacts of such toxin-encoding transgenes on population levels of competitors, preys, hosts, symbionts, predators, parasites, and pathogens (Losey *et al.*, 1999; Poppy, 2000; O'Callaghan *et al.*, 2005; Oliveira *et al.*, 2007);

- interactions and influences of transgenes and GM plants on biodiversity, ecosystem functions, and soil microbes, including target organisms (Kowalchuk *et al.*, 2003; Bellon and Berthaud, 2004; Giovannetti *et al.*, 2005; Oliveira *et al.*, 2007);
- transgene escape to crop landraces and wild relative species through gene flow and its potential (direct and indirect) ecological consequences (Wilkinson *et al.*, 2000; Rieger, 2001, 2002; Snow *et al.*, 2003; Lu and Snow, 2005; Wang *et al.* 2006; Mercer *et al.*, 2007; Rong *et al.*, 2007); and
- potential risks associated with the development of resistance to biotic resistance-transgenes in target organisms (Bates *et al.*, 2005; Dalecky *et al.*, 2007; Li *et al.*, 2007; Wu, 2007).

In addition, there are still some unknown involvements in biogeochemical processes, as well as other potentially significant interactions between transgenic traits and the environment, all of which need to be clearly determined (Heinemann, 2007).

Among the above environmental biosafety issues, transgene escape from a GM crop variety to its non-GM crop counterparts, particularly to the crop landraces and traditional varieties, or to the weedy/wild relatives of crop species has aroused tremendous debate worldwide (Ellstrand *et al.*, 1999; Ellstrand, 2001, 2003; Lu and Snow, 2005; Wang *et al.*, 2006). This is because transgene escape can easily occur via gene flow and may result in potential ecological and biodiversity consequences if significant quantities of transgenes constantly outflow to non-GM crops and weedy /wild relative species. This is particularly true when specific transgenes can introduce evolutionary selective (dis)advantages to the crop varieties or wild populations. The most relevant questions relating to transgene outflow and its potential environmental consequences should be addressed and analysed scientifically in order to have an objective understanding of this issue. The author believes that the full understanding of potential biosafety problems, including transgene escape and its environmental consequences, along with effective assessment and management of such problems, will facilitate the promotion of the further development of transgenic biotechnology, as well as guarantee the safe and

sustainable utilisation of biotechnology and its products in our generation and generations to come. The most relevant questions regarding transgene escape and it environmental consequences include those as listed here:

- Will transgene escape from a GM crop considerably influence the sustainable and safe use of crop biodiversity and impact agro-ecological systems?
- How does transgene escape to non-GM crop varieties and to weedy/wild relatives happen in reality?
- How could escaped transgenes actually affect the genetic diversity of crop landraces and wild populations?
- What are the potential biosafety consequences caused by gene flow from an environmental perspective?
- How can we assess the potential environmental risks caused by transgene outflow using a biosafety framework?
- Can we mitigate environmental risks, if any, through the use of management measures?

These questions should and can be addressed, not only for the benefit of scientists and researchers, but importantly also for the public and consumers of biotechnology products within the international community. The objective of this review is to introduce: the concept and mechanisms of gene flow; the categories of possible consequences caused by pollen-mediated transgene outflow; the biosafety assessment framework for such consequences; and the theoretical means to mitigate and manage any potential environmental consequences, based on knowledge generated from past and current biosafety research.

2. WHAT IS GENE FLOW? - CONCEPT AND BACKGROUND

Gene flow is a natural process that contributes to species evolution. However, in the particular case of GM crops, the flow of one or more transgenes could have adverse environmental, socio-economic, or ethical impacts. Transgene flow from a GM crop to its non-GM crop counterparts (particularly conventional varieties) or to a population of weedy/wild relatives has been considerably discussed as one of the central ecological or environmental risks associated with the application of transgenic biotechnology to crop production (Committee on Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment, 1989). Such environmental risks include potential adverse effects on natural biodiversity and the survival of wild populations. Assessing the consequences

caused by transgene flow is challenging, because it is difficult to predict the ecological effects of transgenes that are integrated into different genetic backgrounds or expressed in different ecological contexts. Indeed, plants that acquire transgenes will continue to evolve, subject to natural and artificial selection pressures in the agricultural setting and beyond. Importantly, once transgenes have moved into new populations, it is impossible to remove them from the environment if the transgenes can successfully persist and spread in the population (Johnston *et al.*, 2008). Therefore, understanding issues such as what is gene flow, what causes gene flow, and what will be the fate of a transgene that has moved into a recipient population through gene flow, is very useful for understanding their potential consequences.

2.1. Definition of Gene Flow

In simple terminology, gene flow is the movement of genetic materials (genes or alleles) from one organism to another. In population genetics, gene flow (also known as gene migration) refers to the transfer of alleles or genes from one population to another (Hartl and Clark, 1989). Theoretically, there are two types of gene flow: **vertical gene flow** and **horizontal gene flow** (Box 1), although the latter is commonly referred to as **horizontal gene transfer**. Horizontal gene flow occurs only among unrelated species, such as between plants and microbes, as well as between microorganisms (Thomson, 2001). The discussion of horizontal gene flow is based more on theory than practice, since it has never been shown to occur with transgenes outside an experimentally-enforced setting, even though this process is significant in the evolution of organisms. Therefore, this review will focus only on vertical gene flow.

BOX 1. DEFINITION OF GENE FLOW

Vertical gene flow – The movement or transfer of genes or alleles by normal reproductive processes, between separate populations of plant and animal species.

Horizontal gene flow – Introduction of genes into organisms by processes that are independent of organism reproduction; infectious.

Gene flow (vertical) is a term extensively used in evolutionary biology and population genetics, long before the issue of transgene escape from GM crops was raised. It is a general understanding that many organisms (mostly plants and animals) are divided into spatially separate populations that have

restricted contact with each other. Spatial separation will possibly lead to reproductive isolation among populations. Many factors can fragment a species into a series of isolated populations. For example, geographical or ecological isolation can cause differentiation of a plant species into populations with different levels of genetic barriers. Conversely, gene flow can also maintain populations with a certain degree of genetic relatedness. Isolation and gene flow are two different forces in the evolutionary process, with the former promoting speciation or diversity, whilst the latter maintains a species with the same genetic identity (Rieseberg *et al*., 2004). Human activity, such as domestication, serves as a strong isolation force that can produce, by selection and cultivation, a separate population derived from a wild plant species. Therefore, gene flow is a natural process that occurs incessantly and permanently between biologically-compatible organisms and to which all genes are subject. In the case of transgene escape, gene flow serves as a medium that moves a transgene from a GM crop to its non-GM counterparts and wild relatives.

2.2. Avenues of Gene Flow

Since gene flow is defined as, for example in plants, the movement of genes from one plant population to another, any medium that can move genes around will lead to gene flow. Typically, there are three avenues for gene flow to be mediated; either by pollen, seed, or vegetative propagule (Box 2, Table 1). **Pollen-mediated gene flow** occurs when pollen grains travel from a plant individual to another individual resulting in fertilisation.

BOX 2. AVENUES OF GENE FLOW

Pollen-mediated gene flow – The movement of genes through pollination between individuals of different populations.

Seed-mediated gene flow – The movement of genes through seed dispersal between different populations.

Vegetative-propagule-mediated gene flow – The movement of genes through dispersal of vegetative organs between different populations.

Table 1. Types of gene flow *via* different avenues and their characteristics

Type of gene flow	Occurrence	Influenced by affinity between donors and recipients	Factors that constrain gene flow
Pollen-mediated	Common	Yes	Outcrossing rate of recipients, pollen loads of donors, pollen competition between donors and recipients, the pollinating media (e.g. wind, animals), and climate conditions
Seed-mediated	Common	No	Seed dispersal media (e.g. wind, water, animals, and humans) and sometimes climate conditions
Vegetative-propagule-mediated (usually for perennial)	Not common	No	Vegetative-organ dispersal media (wind, water, animals, and humans)

This process can happen between individuals within the same population or among separate populations. In the latter case, wind, animals, water current and other factors can serve as media. **Seed-mediated gene flow** occurs through the natural dispersal of seeds by animals, wind, water, or other means from one population to another. Animals with long-range migration habits can transfer seeds over very long distances. Humans can also move seeds intentionally through seed-exchanging and trading within or between geographical regions, which can promote significant amounts of gene flow. The frequencies and patterns of human-influenced seed movement require sociological (seed exchange and distribution) and economic (regional and international trading) analyses and cannot be predicted using only knowledge related to plant biology. In the case of **vegetative-propagule-mediated gene flow**, the movement of genes takes place through the natural dispersal of vegetative organs (e.g. tillers, roots, tubers and rhizomes) of plant species by animals, wind, water, or

other means. As for seed-mediated gene flow, the movement of vegetative organs, particularly by animals and humans, is difficult to estimate when based only on plant biology.

Pollen-mediated gene flow will be primarily determined by intrinsic biological features, particularly the pollination biology of the plant species, such as breeding systems, out-crossing rates, amount of pollen (pollen load) produced by pollen donors, and pollen competition between donors and recipients (B.-R. Lu; unpublished gene flow modelling data). Physical or environmental conditions, such as distances between pollen donors and recipients, the strength and direction of wind, temperature, light intensity, and air humidity, will also influence pollen-mediated gene flow to a great extent. It is very important to generate such baseline biological and physical data through a science-based approach for the accurate prediction of pollen-mediated gene flow. In agricultural ecosystems, humans can play an important role in seed and vegetative-organ dispersal and migration, as would be the case of seeds or vegetative organs falling on the ground during harvesting and picking, transportation to the processing manufacturers, and trading at the local, regional and international level.. The intensity and avenues of gene flow in different crop species can vary significantly, depending on annual or perennial characteristics, the capacity for seed dormancy, the longevity of seeds or vegetative propagules during storage (under natural or artificial conditions), differences in breeding (mating) systems, the importance of such crops in national and international markets, and those parts of the crop that are consumed by humans. Given the complexity of gene movement through seeds or vegetative organs, seed-mediated gene flow and vegetative-propagule-mediated gene flow will not be discussed further in this review, but it is necessary to point out that these are very important avenues for gene flow in terms of evolutionary processes or GM-related biosafety issues.

2.3. Transgene Escape through Pollination, Hybridisation and Introgression

Transgene escape indicates a process in which a transgene(s) moves from a GM crop to its non-GM crop counterparts or to its wild or weedy relatives through gene flow. The escape of transgenes can be categorised into two major types, based on the gene flow avenues through which the transgenes have moved and the recipients. The first category concerning the various available gene flow avenues was discussed in the previous section. For the second category, transgene flow can usually be determined as **crop-to-**

crop gene flow, **crop-to-weedy gene flow**, and **crop-to-wild gene flow** (Box 3), according to the type of recipient.

BOX 3. RECIPIENT-BASED TYPES OF TRANSGENE FLOW

Crop-to-crop transgene flow – Transgene movement from a GM crop to its non-GM crop counterpart.

Crop-to-weedy transgene flow – Transgene movement from a GM crop to conspecific weeds.

Crop-to-wild transgene flow – Transgene movement from a GM crop to wild relative species.

However, what is important to emphasise is that no matter which type of plant is the recipient, non-pollen-mediated gene flow only results in the physical movement of GM seeds or vegetative organs/propagules from one location to another. There is no **hybridisation** or **introgression** (Box 4) involved. Only pollination will cause hybridisation or further introgression of a transgene into wild populations, which will lead to different ecological and evolutionary consequences. It is therefore important to bear in mind that pollen-mediated gene flow is the first, but important, step in the persistence and spread of a transgene in wild populations. For example, pollen-mediated gene flow can produce a hybrid between cultivated rice and common wild rice, and further backcrosses between the hybrids and wild individuals will stimulate the spread of crop genes (including a transgene) in a wild rice population through introgression (Figure 1).

BOX 4. POPULATION ECOLOGY TERMINOLOGY

Hybridisation – A mating between individuals of different populations or closely related species, which usually results in offspring with dissimilar genetic background from the pollen donors and pollen recipients through genetic recombination of genes during hybridisation processes.

Introgression – The stable integration of a gene(s) into a related plant genome via consecutive backcrossing after hybridisation between the two related species has taken place.

Fitness – In biology, fitness means the extent to which an organism is adapted to or able to produce offspring in a particular environment. Usually, the most fit individuals leave the greatest number of offspring. For example, the estimation of fitness for a plant can be made based on its ability to survive and reproduce.

Volunteer – In agricultural rotations, self-set plants from the previous crop that become established as weeds in the current crop. For example, volunteer winter wheat will germinate in a following oilseed rape crop and become a weed.

Persistence – The tendency of an organism to remain in a particular setting over time after it is introduced. A plant displaying persistence is difficult to eradicate from an area once it is planted.

Invasiveness – The ability of an organism to spread beyond its introduction site and become established in new locations where it may provide a deleterious effect on other organisms already existing there.

Selective sweep – The reduction or elimination of variation among the nucleotides in neighbouring DNA of a mutation as the result of recent and strong natural selection. Natural selection will favour individuals that have a higher fitness and, with time, the newly mutated variant of the gene will increase in frequency relative to other variants (alleles) of the gene.

Figure 1. Panicles of cultivated rice (Oryza sativa L., right), common wild rice (*O. rufipogon* Griffin, left), and the hybrid or introgressed progeny (middle) resulting from pollen-mediated gene flow.

Usually, pollen-mediated gene flow is a two-directional event under natural conditions (Lu, 2003). In other words, a crop gene can transfer to weedy and wild species, but a weedy or wild gene can also move to crop species (Figure 2).
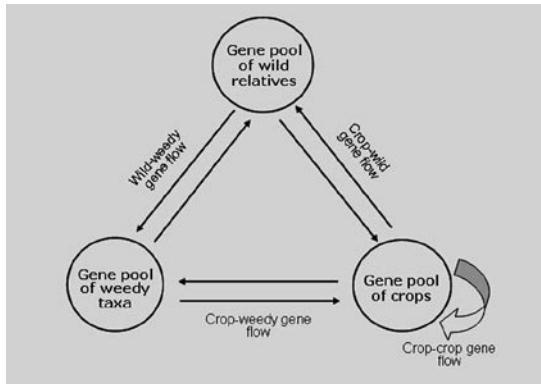

Figure 2. Schematic illustration showing two-directional gene flow among cultivated plant species, weedy types, and wild relatives.

Such gene flow has been found to occur not only between different varieties of the same species (crop-to-crop), but also between crop species and their weedy/wild relatives (crop-to-weedy or crop-to-wild) (Figure 1). Occasionally, gene flow has also been found between a crop and less related wild species (Jenczewski, *et al.*, 2002; Heinemann, 2007) in the same taxonomic genus or different genera, as reported in the wheat grass tribe (Triticeae; Lu, 1993). The direction of gene flow among a crop species, a weedy type, and a wild species can vary considerably, depending on the out-crossing rate of the pollen recipients.

In order for any transgene to escape (through pollination) and spread to wild populations, successful spontaneous hybridisation must happen between a sexually compatible crop plant and recipient species (for example, wild or weedy plants). In general, sexually compatible plants are usually members of the same species or closely related species. If hybrids between transgenic crops and weeds or wild plants produce viable seeds that develop into fertile plants, the offspring may cross with the weedy relative resulting in the introgression of the transgene into the weedy or wild population. The transfer or introgression of transgenes into subsequent generations will depend on the fertility of the hybrid progenies and the selection pressure on the recipient plants hosting the resident transgenes (Chamberlain and Stewart, 1999). Crop plants could also receive pollen from wild or weedy relatives resulting in possible hybrids for generation and dispersal. In principle, there will be no genetic recombination between GM seeds/propagules and those of non-GM crops or wild relative species if no further hybridisation and introgression occur following the physical movement of the transgenes. In this case, the environmental consequences caused by such gene flow may not be so serious, because the transgene(s) will not be integrated into the genomes of the recipient populations. However, if transgene flow mediated by pollination happens, it usually results in hybridisation between the pollen donors (in this case, a GM crop) and pollen recipients (non-GM crop counterparts or weedy/wild relatives) and further introgression.

Hybridisation involves pollination and fertilisation of recipients by pollen donors. Transgenes that are most likely retained in a population of wild relatives are those that enhance **fitness** (Box 4) and increase the ability of the transgenic hybrids to compete in the population under natural conditions in the ecosystem. The fitness performance of offspring resulting from hybridisation in the absence of transgenes can be used as proxies to help the prediction of possible ecological

consequences. In many cases, hybridisation produces offspring as equally fit as their wild parents, which has been extensively reported in spontaneous or artificial hybrids (Arriola and Ellstrand, 1997; Song *et al.*, 2004b; Heinemann 2007). Hybridisation can sometimes produce superior offspring due to hybrid vigour. For example, spontaneous hybrids between cultivated and diverse wild sunflower showed increased fitness in hybrid populations, as measured by Mercer *et al.* (2006) and Baark *et al.* (2008). Sometimes, hybridisation leads to out-breeding depression (Hails and Morley, 2005). Continual replenishment of the environment with a crop that incurs a cost on hybrids derived from crosses with wild populations could, in time, drive the most frequent recipients of the gene into local decline, or even into extinction. As indicated by Hails and Morley (2005), ten worldwide cases involving crops and their wild relatives showed extinction or genetic assimilation of the wild species as a result of hybridisation with the crops. Any increased fitness of transgenic crops will increase the likelihood of transgene introgression into wild populations over evolutionary time by making it easier for crop individuals to persist in natural communities. Stewart *et al.* (1997) observed that *Bt*-transgenic *Brassica napus* demonstrated superior over-winter survival when selection pressures were exerted by insects. If transgenes make a direct contribution to fitness increase (vigour) or decrease (depression), then the environmental consequences is specific to GM crops (Heinemann, 2007). Fitness advantages will "drive" gene introgression, eroding the purity of existing wild species by increasing the number of individuals with those genotypes that benefit from early incorporation of the (trans)gene (Ellstrand, 2003).

Hybridisation between a GM crop and a non-GM crop or a wild relative can occur in one generation, from which the escaped transgene(s) may then integrate into the genome of the non-GM crop varieties or wild relative species through further introgression, resulting in the gradual integration of the transgene into a related plant genome (wild relatives) through consecutive backcrossing. Hybridisation and introgression will promote the long-term persistence and dissemination of transgenes in populations of wild or weedy populations, and may cause unwanted ecological and environmental consequences. In following, pollen-mediated transgene outflow may pose more significant environmental consequences than either seed-mediated or vegetative-organ mediated gene flow because of subsequent genetic recombination and introgression of the transgenes in populations of crops and weedy/wild relatives.

From the viewpoints of population genetics and evolutionary biology, a transgene can be introgressed into a population when the gene makes no contribution to fitness, even in rare cases where it may reduce fitness. However, the likelihood that a transgene will introgress into a wild population is greatly increased when there is a small selective advantage to the recipient plants. Hails and Morley (2005) indicated that the ratio at which genes move into a new population will depend upon the relative fitness of the first hybrids and the progenies of their subsequent generations. It is important, but difficult, to accurately measure a selective advantage or disadvantage of a transgene under diverse conditions. Thus, estimations of the effect of a transgene in new genetic backgrounds, such as in different populations of wild relatives, and under different environmental conditions may not be easily extrapolated from its known purpose and function.

3. POTENTIAL CONSEQUENCES OF POLLEN-MEDIATED TRANSGENE FLOW

From the above, it is easily understood that gene flow is a natural process, and a part of evolution that happens all the time. Therefore, gene flow *per se* is a widespread and natural event that should not generate any potential unwanted ecological risk. This is simply because such a process has been undertaken for many thousands of years during the evolution of vascular plants (higher plants). Even for gene flow between the major domesticated crop species and their wild relatives, such a process has continued for at least a few thousand years, from the onset of the domestication of wild ancestral species into cultivated forms by humans. To date, there has been no report of any major negative environmental consequences or disasters caused by such gene flow.

However, if a gene confers a sufficiently strong fitness advantage, like the case of transgenes with high expression levels and a unique function (such as the insect-resistant *Bt* transgene), it might spread quickly through crop-weedy or crop-wild hybrid progenies in the weedy and wild populations via hybridisation and introgression. Even with very low frequencies of gene flow from GM crops, a transgene can rapidly accumulate and disseminate in weedy and wild populations under favourable selection (e.g., Ellstrand, 2003; Snow *et al.* 2003; Lu and Snow, 2005). It is important to consider that seed-mediated gene flow can also be very extensive in both crop cultivars and their weedy forms due to intentional transportation and exchange of seeds by humans between regions. Strategies for confining the spread of certain transgenes

need to consider the dispersal and longevity of pollen grains in air and seeds in transportation and storage. The estimation of the potential consequences caused by transgene escape, particularly into populations of wild and weedy species, should take the selective advantage of the specific transgenic trait into consideration.

The consequences caused by transgene escape into different recipients can vary significantly in terms of categories and magnitudes. Transgene escape from GM crops to their non-GM counterparts will have completely different affects compared with the escape to weedy and wild relative species. Even in the latter case, different types of transgenic traits will have different effects to wild populations under different environmental conditions. In principle, only transgenes that provide a selective advantage in the evolutionary process and that can change the fitness of the recipient individuals or populations will persist or quickly spread in the wild or weedy recipient populations following transgenes flow. Therefore, the case-by-case principle should be applied rigidly for biosafety assessments of transgene escape and its environmental consequences, and be dependent upon the types of recipients (e.g., crops or wild species) that may incorporate the transgene through gene flow. The potential or predicted consequences caused by different types of transgene flow are described next.

3.1. Crop-To-Crop Gene Flow

Gene flow from one crop field to other adjacent fields planted with non-GM crop varieties of the same species can easily happen. The frequencies of transgene movement mediated by pollination between GM and non-GM crops depend essentially on the breeding (mating) systems and pollen quantity of the crops. Relatively high gene flow frequencies will be expected in out-breeding crops at the same spatial dimension from a pollen source under the same climate condition compared with autogamous crop species where low gene flow frequencies will be expected. On a very practical level, an understanding of crop-to-crop gene flow through both pollen and seeds is useful if different growers or countries want to separate GM crops from their non-GM varieties for marketing or regulatory reasons. This will help to determine the extent of consequences caused by crop-to-crop gene flow in different crops species. For example, cultivated rice is characterised by high rates of self-pollination and very little cross-pollination between adjacent plants or fields (typically less than 1.0 %). Experiments in Italy showed that pollen-mediated gene flow from a transgenic, herbicide-resistant rice variety to

adjacent plants of a non-transgenic counterpart was 0.05 %-0.53 % (Messeguer *et al.*, 2001). Likewise in China, the average frequency of transgene flow from insect-resistant GM rice varieties and their non-GM counterparts was 0.02 %-0.80 % when the plants were grown at close spacing (Rong *et al.*, 2005). Earlier, similar studies also indicated that gene flow frequencies between hybrid rice and traditional landraces grown next to each other were significantly different, with a much higher frequency of gene flow to the hybrid rice (Rong *et al.*, 2004). This asymmetric pattern of gene flow in rice suggests that the frequency is determined by the out-crossing rates of pollen recipients. A further study has shown that gene flow frequency dramatically reduced with the increase of spatial isolation distances from the GM rice pollen donors by only a few metres (Rong *et al.*, 2007). These findings are consistent with the small isolation distances that are recommended for maintaining the purity of cultivated rice grown in seed nurseries. In the USA, for instance, rice plants that are grown for certified seed to be sold to farmers must be isolated from other rice varieties by only 6 metres or less (Gealy *et al.*, 2003). Consequences caused by crop-to-crop gene flow in a cross-pollinating species like maize would be much more serious. This is reflected by the world debates caused by the "contamination" of traditional maize varieties in Oaxaca, Mexico (Quist and Chapela, 2001; Ortiz-Garcia *et al.*, 2005; Raven, 2005).

There are quite a number of predictions related to the consequences of crop-to-crop transgene flow, with only a few included below.

3.1.1. Contamination of non-GM crops

When transgenes move from GM crops to their non-GM crop counterparts, either through seed-, vegetative organ- or pollen-mediated gene flow, a major concern is the "adventitious mixing" (so-called "contamination") of GM and non-GM crop varieties. If the transgene or derived product becomes present in seeds or vegetative organs of non-GM crop to be consumed by human or used as animal feed, such a "contamination" may arouse food and feed safety concerns if the transgene is designed to alter the composition of food crops. There are already a few examples where the products of non-food GM crops have been found mixed with food and feed crops. One of the well known examples is the Starlink™ GM corn (transformation event CBH-351; OECD unique identifier ACS-ZMØØ4-3) that was only approved for use in animal feed. In 2000 the *Bt* (Cry9C) toxin from Starlink™ corn was detected in taco shells, sparking a whole-scale product recall. After three years of intense efforts to recall the seeds, the transgene was still present in detectable

concentrations in the USA food supply (Heinemann, 2007). A recent evaluation strongly suggested that the amounts of Starlink™ corn still in circulation are underestimated and that there is no convincing data to suggest that present levels of "contamination" are reducing further (Marvier and Van Acker, 2005). Another example is the herbicide resistant long grain GM rice (transformation event LLRICE601; OECD unique identifier BCS-OSØØ3-7) from the USA that was found in a number of European countries, as well as in Asian and African countries before it was approved for commercialisation (http://www.foeeurope.org/GMOs/rice_contamination.htm; http://www.radiomundoreal.fm/rmr/?q=en/node/20338). Recently, it was also found in China where GM rice is yet to be approved for environmental release and commercialisation in the market by the Chinese authorities.

These incidences of "contamination" of non-GM crops by GM crops illustrate the problems of crop-to-crop gene flow, as well as the challenges to maintaining segregation between GM crops and their non-GM counterparts. "Contamination" of non-GM crops, particularly organic agricultural products, usually creates social-economic and ethical biosafety concerns. Extensive and long-distance crop-to-crop gene flow will alter the deployment of GM and non-GM crop cultivation. The "co-mingling" of GM seeds or vegetative organs in non-GM crop varieties will cause disputes in regional or international trading, and may well cause legal disputes (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). A significant amount of gene flow to non-GM crops has the potential to increase opportunities for subsequent gene movement to weedy or wild rice populations. In these cases, the level of "mixture" or "contamination" from GM crop by gene flow is crucial. Different countries have set up a threshold to allow defined low levels of the adventitious presence of GM products in non-GM products, providing that the GM products are legally permitted to be commercialised in the exporting countries.

3.1.2. Change of genetic diversity of traditional crops
There is great concern that the extensive cultivation of GM crops will pose potential threats to the genetic diversity of traditional crops (Engels *et al.*, 2006). The loss of genetic diversity of crops in general will reduce the capacity to breed more productive and stress-resistant crop varieties. The concerns of genetic erosion are two fold. First, the extensive adoption of GM crops may lead to rapid losses of traditional crop varieties because of the continuous replacement of the traditional varieties by more commercially advantageous GM varieties. For example, after only a decade of adopting

GM cotton, the current cultivation area of insect-resistant GM cotton (*Bt*) comprises more than 70 % of the total cotton cultivation area in China, and more than 40 % of the total cotton cultivation area in India (James, 2006; Wu *et al.*, 2007). A similar situation is found with GM soya bean and GM oilseed rape in northern America. However, the counter argument is that losses to genetic diversity in traditional crop varieties have already taken place prior to the introduction of GM crops into modern agriculture, with its landscape fragmentation, adoption of improved crop varieties, and degradation of the agricultural environment. Second, the spread of transgenes from a GM crop variety to non-GM traditional varieties through gene flow may change the integrity of the traditional varieties if the transgenes have a selective advantage. During the process of cultivation and seed production, hybrids containing any beneficial transgenes may gradually accumulate unintentionally during selection to ultimately replace the original genotypes of the traditional varieties.

3.1.3. Change in farming practices

Some GM traits require specific management practices to remain effective. For example, the recommended management of a GM *Bt* crop is to have a high dose/refuge strategy, meaning to have a high dose of *Bt* toxin in the GM crops and to have a defined proportion of non-GM plants, capable of hosting the targeted pest species, sufficiently close to the *Bt* crops to allow mating of pests between the two locations. Extensive gene flow creates potential heterogeneity of traits in an environment and will likely change the concentration of *Bt* toxin in populations of GM crops, promoting the evolution of resistance to *Bt* among insects and lowering its strength and the effectiveness of high-dose/refuge strategies (Bates *et al.*, 2005). This can be accelerated by the practice of saving seeds that may lead to heterogeneous mixtures of *Bt* and non-*Bt* plants in subsequent generations (Fitt *et al.*, 2004). Insect-resistant plants are grown on approximately 12 million hectares globally, and adoption is growing (Bates *et al.*, 2005; Marvier and Van Acker, 2005). Moreover, the range of plants with *Bt* variants available or being developed is expanding, and includes cotton, maize, tomato, aubergine (otherwise known as eggplant or brinjal), soya bean, oilseed rape, potato, apple, peanut (groundnut) and broccoli (Bates *et al.*, 2005). This undoubtedly extends the variety of insects that will one day be exposed to *Bt* toxins, with the possible consequence that any management failures could extend to multiple crops and countries in a short period of time (Heinemann, 2007).

Another consequence brought about by gene flow is the creation of a landscape mosaic. Residual plants expressing *Bt* toxin-derived insect resistance, whether they be volunteer or feral crops or wild relatives, could undermine even region-wide efforts to coordinate *Bt* cropping practices. Stacked varieties might not significantly improve the management of *Bt* resistance if they are introduced after the same toxins have been individually introduced through commercialised varieties or if the toxin genes separate during transgene flow. The extensive cultivation of herbicide-resistant GM crops will also accelerate changes in farming practices by requiring the use of selective herbicides, thereby replacing traditional weeding practices that utilise broad spectrum herbicides or manual weeding, for example (Lu, 2006).

3.1.4. Issues with pharmaceutical and industrial GM crops

One of the main concerns with crop species modified to produce pharmaceuticals is that they could harm humans or other species when accidentally consumed following unintentional "co-mingling" with conventional crops in the food chain. Some GM crops have been modified to serve as "bioreactors" for the production of pharmaceutical products and industrial chemicals, such as the GM rice that produces medicine (Oszvald *et al.*, 2007). These GM crops are expected to be unsuitable as food for human consumption because they have no historical record of safe use (Heinemann, 2007). Gene flow from such GM crops into the general human food chain would certainly create adverse effects and greater concerns. The hazards will relate directly to the original transgenic crop. For example, a gene for the production of a vaccine protein may be transferred to a non-GM crop through extensive gene flow, with the same spectrum of concerns surrounding either the original or the hybrid crop entering the human food supply (Heinemann, 2007). Alternatively, novel hazards might arise from transgene flow, because the expression of a protein in one food crop may be significantly different from its expression in another. This was illustrated when a protein from a bean with a history of safe use as human food was demonstrated as being a potential allergen after it was transferred and produced in peas (Prescott *et al.*, 2005). Care must also be taken to ensure that future GM crops specifically modified to have altered nutritional levels, such as the so-called "Golden Rice" (Ye *et al.*, 2000), will be safe for consumption by the general populace should they enter the human food chain in an unfettered manner.

3.2. Crop-to-Wild Gene Flow (Including Crop-to-Weedy Gene Flow)

Ecological studies have shown that some GM crops are viable in natural

ecosystems and can interbreed with their wild relatives. The most publicised environmental consequence is that invasive weeds may be created if GM crops modified to tolerate herbicides or to resist diseases and pests transfer their transgenes to wild or weedy relatives *via* gene flow. Plants can also be modified with traits that allow them to grow faster (for example, by expressing a specific growth hormone), reproduce more (for example, by enhancing seed production), and live in new types of habitats (for example, by enhancing drought and cold tolerance). In principle, the potential environmental consequences caused by crop-to-wild or crop-to-weedy transgene flow can be effectively determined by the amount of transgenes that have outflowed to the wild and weedy populations, and by the characteristics of the GM traits that have or do not have evolutionary advantages under natural selection. When populations incorporate a GM trait likely to confer a selective advantage and are then exposed to a relevant selective pressure (e.g., pest attacks or drought/salinity stresses), the populations will most likely exhibit an enhanced performance (Ellstrand, 2003; Lu and Snow, 2005) leading to unwanted environmental consequences. It is necessary to point out that crop-to-wild or crop-to-weedy gene flow can recur over time. This is because plants of wild and weedy species generally persist in their habitats, or their seeds remain in the local soil seed-bank. The frequency of transgene flow can increase through recurrent gene flow over different years/seasons from GM crops cultivated in surrounding area. This is different from the case of transgene flow to cultivated species that are harvested at the end of the season. If the crops are consumed or used by industry/manufacturing, the transgenes do not accumulate in the crop populations. However, if the crops are to be used as seeds, the contaminated seeds may be propagated and disseminated to different regions.

Because of these unique features, the direct and immediate consequence of crop-to-wild gene flow will be changes in the genetic integrity of wild or weedy species by recurrently pumping transgenes into wild populations. In addition, if the transgene conveys a selectively advantageous trait(s), the movement of such a transgene into wild relatives may change the fitness of wild or weedy relatives, resulting in demographic alterations (either increase or decrease) of the wild and weedy populations, or rapid accumulation and spread of the transgenes in the populations by speeding up introgression of the transgene into wild or weedy populations. These changes will potentially lead to diverse environmental consequences.

There are different possibilities for the fate of a wild population which incorporates a transgene. On the one hand, if the transgene can enhance the fitness of wild relatives through the expression of a favourable trait such as pest resistance, drought tolerance, or enhanced growth ability, the outflowed transgene will persist and quickly spread in the population through introgression. The individuals that express the transgene will out-compete those individuals without the transgene under natural selection. This process will promote a rapid <u>increase</u> of transgenic individuals and enhance their invasiveness, causing different degrees of weed problems by enabling the wild populations to expand into new territories. With the advent of herbicide resistant crops, oilseed rape being the most well known, there is strong public concern about the production of "super-weeds" that are resistant to multiple herbicides.

On the other hand, if the transgene <u>reduces</u> the fitness of receiving wild relatives, the frequencies of individuals that contain the disadvantageous transgene will decrease gradually. This process will be accelerated by recurrent gene flow and introgression from the nearby GM crop, possibly leading to the extinction of local populations by the so-called swarm effect (Ellstrand and Elam, 1993). In many parts of the world, such **swarm effects** have already happened in the absence of GM crops through crop-to-wild gene flow (Kiang *et al.*, 1979), where populations of wild relatives are surrounded by crop fields in agricultural ecosystems and inhabit bordering areas between agricultural lands and natural habitats (Wilkinson *et al.*, 2000; Ellstrand *et al.*, 2007).

If the transgene is selectively neutral and does not alter the fitness of the wild relatives, such as those genes encoding nutritional compounds and quality traits that are only favourable to human taste or health conditions, the outflow of such a transgene may not have considerable influences on the population dynamics of the wild relatives. In this case, the likelihood of environmental impacts of such gene flow should be low.

Environmental consequences caused by crop-to-wild or crop-to-weedy gene flow are profound and need to be determined in the long-term. There are still many biological mechanisms underlying the process of gene flow and fitness change to be understood. Listed here are only some of the hypothesised/predicted consequences of crop-to-wild gene flow that are commonly discussed and debated worldwide, although most have never been found or proven. Science-based studies should be conducted to test whether in

reality such consequences will happen under a case-by-case situation, and to measure the magnitude of such consequences should they occur.

3.2.1. Creation of new weeds

Weeds can cause significant yield loss to a crop if the weed populations extensively infest agricultural fields, competing for resources with the crop (Hoagland and Paul, 1978). In addition, weeds can also lower the commercial quality or nutritional value of a crop by introducing undesirable grains, toxins, or allergens (Kwon *et al.*, 1991), whether they are sourced naturally or from GM plants. Any transgene-expressing weeds or wild populations are expected to create the same serious management problems as those currently experienced with their non-transgene-containing counterparts.

Crop-to-wild transgene flow may accentuate the characteristics of weediness, leading to greater **persistence** and **invasiveness** (Box 4) of already existing weeds. On the other hand, a GM crop may acquire genes for weediness leading to persistence and invasiveness of a crop species. Therefore, the concerns of gene flow with respect to weediness are mostly related to the following two aspects: (1) a wild or weedy species, for example a wild and weedy rice or oilseed rape that invades and persists in crop fields has the ability to become a more effective and aggressive weed; and (2) a GM crop **volunteer** (Box 4) or hybrid between the GM crop and wild relatives has the ability to become a more effective and aggressive weed, after incorporating transgenes that convey traits against biotic and abiotic stresses. These concerns relate to the hypothesis that a transgene from GM crops will bring a fitness advantage to the populations of crop volunteer, weeds, and wild species (Ellstrand *et al.,* 1999; Ellstrand, 2001; Lu and Snow, 2005).

Some noxious weeds, such as charlock (*Sinapis arvensis*) in the UK, have seeds that can persist in soil for up to 35 years. This observation is important due to the detection of a hybrid of charlock and herbicide-tolerant oilseed rape in a large UK study. While the seeds of the hybrid did not germinate, the pollen of the plant was not tested for the presence of the transgene (Daniels *et al.*, 2005). Thus, transgene flow could potentially make this important weed herbicide-tolerant. Traits that may influence invasiveness include fertility, vegetative vigour, tolerance of a wide range of environmental conditions, and the quality and dispersal range of viable material. Gene flow between crops and their weedy populations is relatively high, because the weedy populations are conspecific with the crops, usually derived from volunteers of

the same crop species, or from offspring of hybrids between crops and their wild relatives (Lu, 2003).

Recurrent or sequential transgene outflow from a GM crop may cause transgene stacking in the same type of wild relatives or volunteers. Cross pollination between varieties of oilseed rape (*Brassica napus*) in Canada has resulted in spontaneous triple-herbicide-resistant variants (Hall *et al.*, 2000). Volunteers of oilseed rape are considered as among the 20 most common weeds in fields in Alberta, Canada, occurring as a residual weed in wheat and barley fields (Hall *et al.*, 2000). Stacked herbicide tolerance in weeds or volunteers can significantly increase difficulties for weed control.

3.2.2. Effects of other fitness-related transgenes

In many cases, transgenes do not encode traits with an evolutionary selective advantage. These types of transgenes are not expected to persist and spread in volunteer/weedy or wild populations of plant species. Therefore, the outflow of such transgenes is unlikely to result in environmental problems because frequencies of the transgenes would remain very low in populations. Likewise, transgenes that confer a fitness cost (in the form of reduced survival or fecundity) will be less likely to be passed on to host progeny (e.g., Gressel, 2000). Many transgenic traits related to nutritional quality, manufacture processing quality, and grain composition are likely to have neutral or even negative effects on the fitness of weedy and wild relatives of the crop. These include for example, the *psy*, *crtI*, and *Gt1* genes that increase the content of vitamin A (in the form of ß-carotene) in golden rice (Ye *et al.*, 2000) and the antisense Wx gene that improves the taste quality of rice grains (Chen *et al.*, 2006). These transgenes may pose limited environmental consequences should they escape to weedy or wild relative species through gene flow.

In contrast to the above cases, the fitness of wild and weedy populations might be enhanced by transgenes that confer a greater resistance to biotic stresses (such as insects and diseases), or a greater tolerance of abiotic stresses (such as drought and salinity), or an enhanced production of seeds or vegetative propagules. Depending on local environmental conditions, the flow of these types of "fitness-enhancing" transgenes to nearby recipients could release weedy or wild populations from ecological pressures that restrict their local abundance or limit their habitat requirements. If the transgene recipient population is already a weed or has the potential to become more invasive by acquiring specific transgenic traits, it might become more invasive in natural

habitats. For example, if wild or weedy rice acquires transgenes that confer salt tolerance and faster growth rates, it will become more invasive in brackish and saline habitats. Another question concerning the consequences of gene flow from a transgenic crop relates to the magnitude of the transgene-derived fitness benefit and whether this will affect the population dynamics. This will largely depend on the extent to which insect and disease pressures regulate the wild relative populations. Therefore, fundamental ecological studies of wild populations are needed to help address this question. In wild sunflower, for example, the expression of a single *Bt* transgene resulted in less damage from insect larvae and a large boost in fecundity in field-grown experimental plants in Nebraska, USA (Snow *et al*., 2003). Therefore, if certain transgenes can enhance the fitness of wild or weedy species, it is important to evaluate 1) the potential for creating more invasive weeds, 2) any possible harm to non-target species, such as beneficial insects, and 3) any possible effects of gene flow on the long-term durability of insect-resistant and glyphosate-resistant crops (e.g., Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants, 2002; Snow *et al*., 2005).

3.2.3. Spread of transgenic herbicide resistance

Transgenic herbicide resistance is the type of trait that could easily be acquired by wild and weedy species through gene flow. Weed control in crop fields is increasingly dependent on herbicides in both developed and developing countries, because of the shortage in agricultural labour and changes in farming practices, for example, the shift from transplanting plantlets to direct-seeding in rice. This transition has resulted in worse problems with weeds, due to weed seedlings out-competing, and suppressing the growth of, rice seedlings (IRRI, 2000), as well as causing a severe occurrence of weedy rice in northeastern China (Cao *et al*., 2006). Rice fields that become heavily infested with weedy rice can become unusable because the weed is an effective mimic of the crop and its longevity in the seed-bank makes it very difficult to eradicate. Thus, rice growers who can afford the cost of herbicides are eager to adopt herbicide-resistant rice varieties, even though the benefits of this strategy could be short-lived. The cultivation of herbicide-resistant rice varieties will certainly complicate the situation of weed control after a short period of time, because resistance to different types of herbicides is inherited as a dominant Mendelian trait that can easily spread to weedy rice by cross-pollination (e.g., Gealy *et al*., 2003). If the same herbicide is used repeatedly, selection favouring the GM herbicide-resistant weedy rice will be very strong. Modelling studies by Madsen *et al*. (2002) estimated that herbicide resistance

may become common in weedy rice populations within only 3-8 years of continuous rice cropping. A similar situation is expected with the cultivation of GM oilseed rape. Different herbicide resistant transgenes were found to be stacked in the same volunteers following recurrent gene flow (Hall *et al.*, 2000), and the same transgenes are also expected to be taken up by any weedy types occurring in the neighbourhood.

Therefore, it seems extremely likely that new genes for herbicide resistance will spread to weedy types and volunteers, especially in regions where weed management is already difficult. In general, it seems likely that most fitness-enhancing traits will not be able to spread to weedy populations as quickly as those genes for herbicide resistance in populations that are frequently exposed to the herbicide in question. Even for self-pollinating wild or weedy species that can incorporate transgenes at very low rates, once transgene flow has occurred, those self-pollinating plants with a greater fitness will quickly increase the number of transgenic progeny in subsequent generations. In Costa Rica, for example, the rapid development of glyphosate-resistant weedy rice derived *via* gene flow from a herbicide resistant rice variety bred from a mutant has already become a major economic problem for rice farmers who rely on glyphosate for no-till rice production and who are not able to implement weedy rice control (Bernal E. Valverde, Tropical Agriculture Research and Development, Alajuela, Costa Rica, personal communication). Consequently, a number of rice scientists have recommended that herbicide-resistant rice should not be widely used without strict stewardship guidelines and effective biological confinement techniques to mitigate transgene flow (e.g., Gressel, 2000; Olofsdotter *et al.*, 2000; Madsen *et al.*, 2002).

3.2.4. Loss of genetic diversity in wild germplasm
Wild relatives of crop species are widely viewed as valuable resources of genetic diversity for future breeding (e.g., Vaughan, 1994; Ellstrand, 2003). Even if these wild relatives are somewhat weedy, germplasm experts believe that these reservoirs of diversity should be protected from population extinction and genetic "swamping," which results from a heavy influx of crop genes. To some people, the mere presence of transgenes in the wild germplasm of crop relatives represents a form of "contamination" or "genetic pollution".

There are two scenarios for the unwanted effects of transgenes on genetic diversity. First, it is theoretically possible that strong selection for fitness-

enhancing transgenes could generate **selective sweeps** (Box 4), in which portions of the crop genome that are linked to these transgenes displace corresponding portions of wild genomes (Ellstrand, 2003; Gepts and Papa, 2003). This process is expected to be more common in self-pollinating species than in out-breeders, which have a greater potential for the mixing and dilution of crop alleles during sexual reproduction. Also, selective sweeps could be favoured by clonal reproduction, which might allow more vigorous transgenic crop-wild hybrids to out-compete the non-GM plants at the local level. The potential for rapid selective sweeps in most self-pollinating plants seems remote because 1) few transgenes seem likely to confer fitness benefits that are strong enough to lead to selective sweeps in wild populations, and 2) the extent of pollen-mediated gene flow is typically very low. However, massive transgene flow to wild relatives through recurrent pollination may pose threats to wild germplasm, particularly for out-breeders. Second, in some situations, a large influx of fitness-reducing transgenes could contribute to population declines or even local extinction of small, isolated populations of wild plants that occur near the crop (Haygood et al., 2003). In populations of 100 individuals or more, frequencies of fitness-reducing transgenes would diminish due to the purifying force of natural selection. Therefore, current information suggests that gene flow from self-pollinating GM crops may not threaten the genetic diversity of wild and weedy relatives to a greater extent than current gene flow from conventional varieties (Ellstrand, 2003; Gepts and Papa, 2003).

Gene flow may affect genetic diversity if it compromises the survival of populations of plants that are valued for not having a particular transgene. Biodiversity may be threatened by the escape and proliferation of a competitive accession expressing the transgene. Perhaps less well recognised is the loss of diversity through genetic erosion. While this is a natural process, GM agriculture can be conducted on scales that significantly distort the normal impacts of genetic erosion through gene flow. Not only are large areas covered in GM crops, but they are replenished frequently by human cultivation, leading to repetitive introductions of the exotic genes into an environment.

4. RISK ASSESSMENT OF POLLEN-MEDIATED TRANSGENE ESCAPE

4.1. Risks and Risk Assessment
Generally, "risk" is defined as the expectation that a threat may succeed and the potential damage or hazard that can occur. Very often, people mix

up the meaning of "risk" and "damage" (or hazard), but correctly speaking, the meaning of a risk is not equal to that of an existing damage or hazard. It is very important to know that risk is the probability or likelihood in which a specific damage or hazard will occur, and that certainty is the special case of risk in which this probability is equal to zero (0 %) or one (100 %). In the context of environmental biosafety, a risk indicates the probability of any damage or harm to the environment as a result of the extensive release of GM plants into the environment.

Risk assessment in general indicates a critical and productive exercise that helps to determine the occurrence and magnitude of relevant risks. The objective of risk assessments is to reduce the risks of exposure to the environment to an absolute minimum level. Risk assessment can be qualitative or quantitative. In the presence of known damages or hazards (e.g., levels of toxicity of a transgene to the environment), quantitative assessments can be done. However, in many cases, quantitative data are incomplete or even absent, which makes the risk assessment exceptionally challenging. Therefore, the determination of quantitative data associated with risks is essential during the risk assessment exercise.

4.2. Principles of Biosafety Risk Assessment
In the context of environmental biosafety, risk is derived as a function of hazard and exposure. Here, **hazard** represents the intrinsic properties of a substance or object (in this case, a transgenic plant or transgene product) with potential adverse or harmful effects. **Exposure** is a quantitative measurement of the extent to which a given hazard is present in a particular dimension (in this case, the environment or ecosystem). Therefore, **Risk** indicates the probability that any adverse effect will occur from an environmental hazard.

The effective assessment of environmental risks created by the extensive release of GM crops depends essentially on the knowledge of potential adverse or harmful effects from a transgenic plant and their probability to occur. Therefore, the establishment of such knowledge is key, prior to the exercises. A biosafety risk assessment usually follows four steps: (1) hazard identification; (2) exposure assessment; (3) effects assessment; and (4) risk characterisation (Andow and Zwahlen, 2006). Therefore, to design a protocol for environment-related biosafety risk assessment, one should consider the key factors and steps that can cause adverse or harmful

effects caused by the cultivation of a GM crop and the potential in which the adverse effects will occur.

5. THE PROCEDURE OF RISK ASSESSMENT FOR TRANSGENE FLOW TO WILD RELATIVES

To effectively assess environmental biosafety consequences created by transgene escape from GM crops to wild relatives through pollen-mediated gene flow, it is necessary for us to attain knowledge (e.g. the baseline data of crops and their wild relatives) that is relevant to the particular biosafety assessment, as well as to determine any knowledge gaps that are essential to address the relevant scientific questions at hand, stringently following the principle of biosafety risk assessment. The knowledge gaps include the following aspects:

1) What is the possibility that a transgene can move from a GM crop to wild relatives, and what is an accurate frequency of such an occurrence?

2) What is the destiny of a transgene that has introgressed into individuals of wild relatives?

3) Can transgenes change the fitness of individuals of wild relatives?

4) Can transgenes alter the demographical dynamics of a wild population?

5) Does expressing a transgene significantly enhance the invasiveness of wild individuals and populations?

The correct answer of each of these questions will certainly help to reduce knowledge gaps and facilitate the establishment of a standard protocol that is useful for assessing gene flow-related environmental risks.

5.1. Setting the Scene: A Framework

As discussed in the previous section, risk assessment is an exercise or a procedure that helps to determine the likely occurrence and magnitude of relevant risks. To meet the objective of a risk assessment, it is necessary to establish a general framework and protocol for determining whether or not environmental risks associated with transgene flow will occur, and how serious the risks will be at the various steps. Through the logical analyses of transgene escape and its potential environmental consequences in the previous sections, it can be seen that there are three major steps or procedures closely associated with the rational assessment of transgene escape and its environmental consequences.

First, it needs to be understood whether or not a transgene could flow from a GM crop to its wild relatives. If no such possibility for gene flow exists, due to biological, temporal, and spatial constraints, there will be no pollen-mediated transgene escape to wild relatives, and consequently, no further risk assessment is required. Second, it needs to be understood whether or not the introgressed transgene(s) will express and inherit normally in the hybrids and advanced progenies between a GM crop and wild relatives. If a transgene can be incorporated into a wild individual or population through gene flow, but cannot express normally to exert its normal function, there should be no, or very limited, environmental consequences after the incorporation of such a transgene into the wild. Accordingly, no further risk assessment is required. Third, it needs to be understood whether or not the introgressed transgene(s) could change the ecological fitness of the wild recipient individuals, and affect the dynamics of a wild recipient population, which may bring about an increase in invasiveness of wild individuals or populations. As illustrated in Figure 3, transgene escape and its potential environmental consequences will essentially depend on the success of a transgene from outflowing to establishing/spreading in a wild population through successive procedures. The potential risks created by transgene flow following this framework can therefore be assessed once necessary baseline data are collected.

Additionally, in biosafety risk assessment, there are a few important principles to follow, such as the science-based principle, case-by-case principle, and step-by-step principle. These principles serve as an excellent guide to effectively undertake the biosafety assessment of the environmental consequence derived from transgene flow in a tiered approach, as indicated in Figure 4.
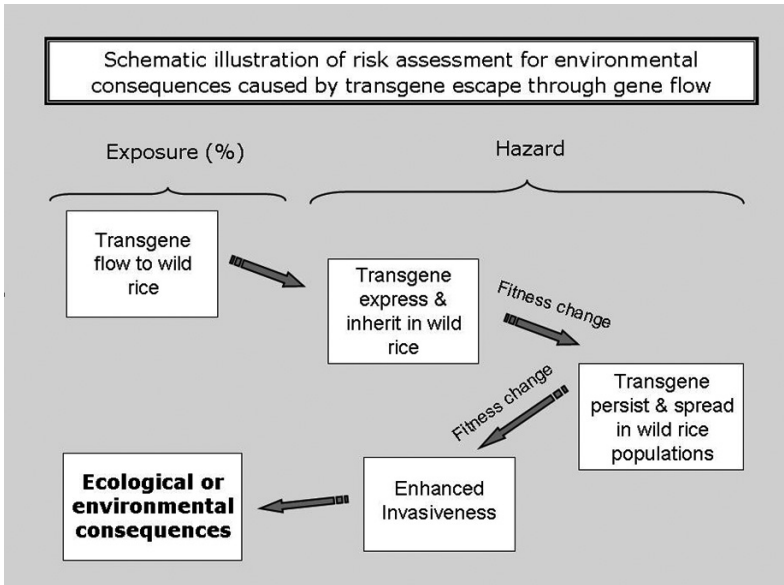
Figure 3. A conceptual framework indicating the three important platforms for risk assessment of transgene escape from a GM crop to its wild relative species through pollen-mediated gene flow and the potential environmental consequences.
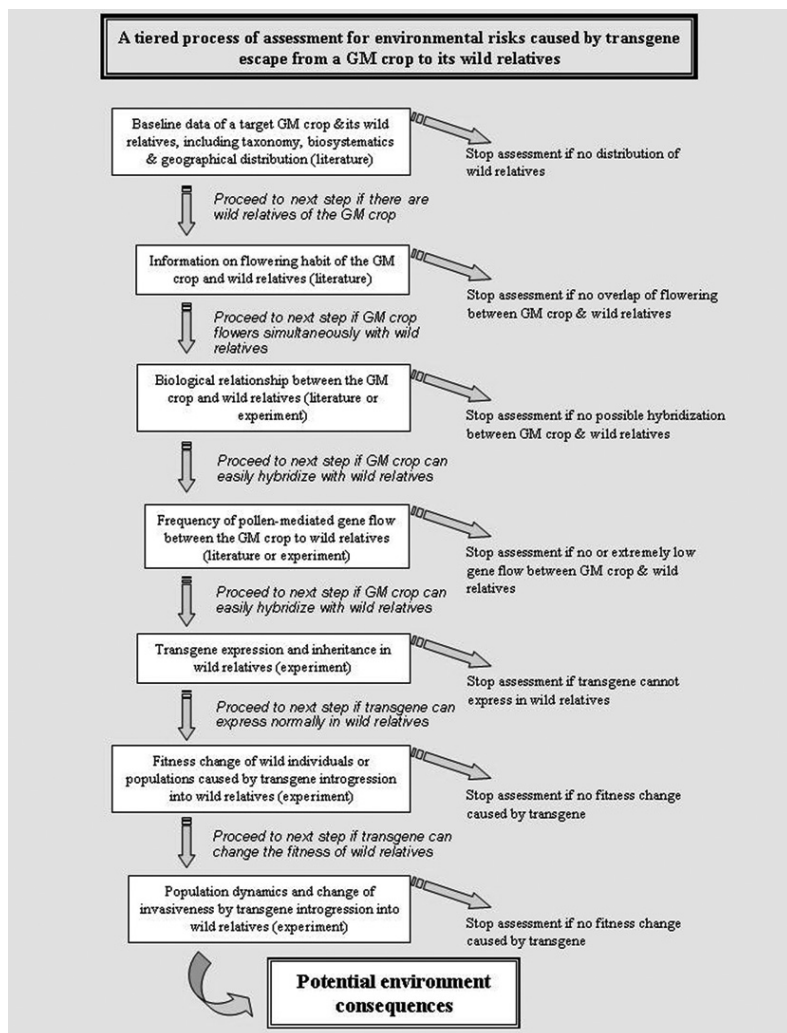
A tiered process of assessment for environmental risks caused by transgene escape from a GM crop to its wild relatives

Baseline data of a target GM crop & its wild relatives, including taxonomy, biosystematics & geographical distribution (literature)
→ Stop assessment if no distribution of wild relatives

Proceed to next step if there are wild relatives of the GM crop

Information on flowering habit of the GM crop and wild relatives (literature)
→ Stop assessment if no overlap of flowering between GM crop & wild relatives

Proceed to next step if GM crop flowers simultaneously with wild relatives

Biological relationship between the GM crop and wild relatives (literature or experiment)
→ Stop assessment if no possible hybridization between GM crop & wild relatives

Proceed to next step if GM crop can easily hybridize with wild relatives

Frequency of pollen-mediated gene flow between the GM crop to wild relatives (literature or experiment)
→ Stop assessment if no or extremely low gene flow between GM crop & wild relatives

Proceed to next step if GM crop can easily hybridize with wild relatives

Transgene expression and inheritance in wild relatives (experiment)
→ Stop assessment if transgene cannot express in wild relatives

Proceed to next step if transgene can express normally in wild relatives

Fitness change of wild individuals or populations caused by transgene introgression into wild relatives (experiment)
→ Stop assessment if no fitness change caused by transgene

Proceed to next step if transgene can change the fitness of wild relatives

Population dynamics and change of invasiveness by transgene introgression into wild relatives (experiment)
→ Stop assessment if no fitness change caused by transgene

Potential environment consequences

Figure 4. An outline of the step-wise process for assessing potential environmental consequences caused by transgene escape through pollen-mediated gene flow following the concept illustrated in Figure 2.

5.2. Baseline Information

The availability of baseline information for a target (GM) crop and its weedy/ wild relative species is very important for facilitating the assessment of the environmental biosafety consequences associated with pollen-mediated transgene escape. Normally, there are three prerequisites that decide whether or not pollen-mediated transgene escape from a GM crop to its wild relatives will happen:

- The first prerequisite is that, *spatially*, a GM crop and its wild relatives should have a sympatric distribution and be grown in close vicinity. This will ensure that pollination between a GM crop and its wild relatives occurs.
- The second prerequisite is that, *temporally*, the flowering time (including flowering duration within a year and flowering time within a day) of a GM crop and its wild relatives should overlap. This means that there should be synchronised flowering of a GM crop and its wild relatives.
- The third prerequisite is that, *biologically*, a GM crop should have a sufficiently close evolutionary or genetic relationship with its wild relative species to guarantee successful sexual hybridisation between the crop and wild plant. In addition, the resultant interspecific hybrids should be able to survive and reproduce normally.

If the three prerequisites cannot be met, usually transgene escape from a GM crop to its wild relatives through gene flow will not happen. Therefore, prior to any risk assessment of gene flow-related consequences to the environment, it is necessary to obtain the baseline information outlined below.

The opportunity for gene flow from a GM crop to its wild relatives will vary considerably from location to location because of variation in the geographic distribution of relatives. The presence of wild or weedy populations in a region may create more concerns than when a region has no close relatives to the crop. Information on the distribution of wild relatives of the target crop should be available through consulting herbaria, and searching the scientific literature. Critical baseline information will be required to answer the following questions:

- What are the crop wild relatives within the area where the GM crop will be released? How many, and how frequently do, wild relative species occur?
- What is the geographical distribution of the concerned crop wild relatives? Are these wild relatives widely grown or endemic to a region?
- Which of these crop wild relatives can outcross naturally with the GM crop?

- Are there conspecific weedy populations occurring in the areas where the GM crop will be released? How abundant and diverse are these weedy populations?
  (Step one in Figure 4)

There are some basic biological features that will lay the foundation for potential gene flow. For example, wind-pollinated species may have higher average pollen movement than species that are self-pollinated or insect-pollinated. In a reported case of wind-pollinated creeping bentgrass (*Agrostis stobnifera L.*), gene flow was detected at 21 kilometres distant (Watrud *et al.*, 2004). Pollen longevity and crop height may also affect the distance that pollen travels. The following biological features are essential for understanding the potential of transgene escape through gene flow:

- What types of breeding (mating) systems do the target crop and its wild relatives have (e.g. obligate out-crossing, strict self-pollination, or mixed mating)?
- What is the flowering habit of the crop and its wild relative species?
- How far does pollen disperse from crop fields and how long does the pollen remain viable?
- To what extent does the crop out-cross with its wild relatives in natural habitats?
- What type of vectors (e.g. wind, insect) lead to pollen movement from the crop?
  (Step two in Figure 4)

The biological relationships of a crop with its wild relatives determine the magnitude of crop-to-wild gene flow and introgression. Even though the GM crop and its wild relatives can hybridise with each other and form spontaneous hybrids, the viability of the F1 hybrids will determine whether the transgene will persist in wild populations. Using results generated from experimental crosses between commercial crop varieties and different types of wild relatives will assist in predicting the potential consequences of gene flow. There are many such studies of crop-wild crosses and hybrid analysis that provide good references for judging the possibility of crop-wild hybridisation and introgression (Linder *et al.*, 1998; Jarvis and Hodgkin, 1999; Ellstrand, 2003; Song *et al.*, 2004b). The following baseline information regarding the ability of survival and reproduction of hybrids are also important:

- Do artificial crosses between the crop and potential gene flow recipient wild relatives easily result in viable F1 hybrids?

- Do crop-wild hybrids arise spontaneously in natural habitats? How abundant do hybrids occur?
- Are F1 hybrids vigorous and fertile under experimental and field conditions?
- Can backcross hybrids be made under experiment conditions? Are introgressed progenies observed under field conditions?
- How fit are the hybrids and introgressed progenies in natural habitats?
  (Step three in Figure 4)

When all the spatial, temporal and biological prerequisites are met, it is then essential to assess actual gene flow frequencies to determine the level of potential environmental risks caused by gene flow.

5.3. Estimation of Gene Flow Frequencies

Estimating frequencies of pollen-mediated gene flow is a key component of, and the primary step in, risk assessment. It will answer questions relating to "exposure" in the risk assessment process. Measuring the frequencies at which gene flow occurs is a challenging task in many crop-wild complexes, because, as discussed in the previous section, gene flow frequencies can vary significantly between plant species with different mating systems and modes of pollination (e.g. wind pollination *versus* insect pollination). Pollen-mediated gene flow is influenced by many biological factors, such as; flowering habits, out-crossing rates, the amount of pollen produced and the duration of pollen viability, the sizes of pollen donor and recipient populations, as well as by many non-biological factors, such as; the distance between pollen donors and recipients, wind speed or insect pollinator activity, humidity, and other climate conditions. Therefore, the measurement of gene flow frequencies for different crop-wild species at different locations should strictly follow the case-by-case principle (Lu and Snow, 2005). In other words, gene flow data obtained for one type of crop (e.g. wind- and self-pollinated) cannot be used for the risk assessment of another type of crop (e.g. insect-pollinated out-breeder). A number of experimental and empirical approaches have been developed to estimate the relative frequencies of pollen dispersal and pollen-mediated gene flow rates of plant species (Lavigne *et al.*, 2002; Ellstrand 2003; Song *et al.*, 2003; Lu and Snow, 2005; Koopman *et al.*, 2007), which can be used for measuring transgene escape from GM crops.

Prior to obtaining crop-to-wild gene flow frequencies, it is very helpful to study

the crossability and compatibility between the crop species that the GM trait will be, or has already been, transferred to and the potential wild relative species that occur in the areas where the GM crop is expected to be cultivated. This assists in determining the various possibilities in which the transgene could move from the GM crop to its wild relative species. If the crop has a high crossability with its wild species under natural conditions, then transgene outflow to wild species/ populations will be high, and *vice versa*. Studies of the crossability between a crop and its wild relative species can be conducted in the greenhouse or field by hand pollination, including reciprocal crosses (using wild relatives as both maternal and paternal parents). The number of individuals used during test pollinations should be sufficient (>30 individuals for each parent) to ensure that the resulting data is truly representative of the crossability between the crop and wild species. If the crop species is not compatible with the wild relatives or the ratio of crossability is extremely low, no further biosafety assessment for transgene escape to wild relatives *via* pollen-mediated gene flow is needed. Otherwise, the biosafety assessment should proceed to the next tier/step. The crop-wild crossability can be estimated from the ratio of seed set (Rs) between the crop and wild species after hand-pollination, which can be calculated from the formula:

$$R_S \ (\%) = \ N_h / T_f \times 100\%$$

R_S = Ratio of seed set; N_h = Number of hybrid seeds obtained; T_f = Total number of flowers pollinated.

It is also important to examine the fertility of the artificial crop-wild hybrids, including pollen (male) fertility and seed (female) fertility. Pollen fertility, ascertained by staining hybrid pollen grains with an iodine-potassium iodide (I-KI) solution, can be calculated from the formula:

$$F_{po} \ (\%) = P_s / P_t \times 100\%$$

F_{po} = Pollen fertility; P_s = Number of stainable pollen grains; P_t = Total number of pollen examined,

whereas seed fertility can be estimated using the following the formula:

$$F_{pa} \ (\%) = S_g / S_t \times 100\%$$

F_{pa} = Seed fertility of hybrids; S_g = Number of good seeds; S_t = Number

of seeds examined

An accurate measurement of the frequency of gene flow mediated by pollination is vital in preliminary estimations of the extent of risks caused by transgene escape to wild relative species, given that the risk is determined by the amount of transgenes that move to wild populations and the adverse effect of the transgene to wild populations. However, the frequency of gene flow can be significantly different among plant species with diverse mating systems, as well as diverse climate conditions. Therefore, gene flow frequencies measured from one species cannot be used for another species, and even the measured gene flow frequencies from one variety of the same species cannot be used completely for other varieties under different environmental conditions. Thus, the "case-by-case" principle should be strictly followed in biosafety assessment.

Information on individual out-crossing rates and variation in flowering times is useful for evaluating the potential for hybridisation. Small-scale experiments involving plants with distinct genetic markers can be used to measure gene flow between adjacent plants in a given location and year, but they may not reflect large-scale or long-term processes. Although these types of information are undoubtedly incomplete, they can be used to assess the potential for transgene escape and to develop strategies to minimise the escape of certain types of transgenes via pollen.

Seed-mediated gene flow can also be very effective as a means of transgene dispersal, especially when seeds are traded within and between countries. Usually, rice seed have their husks removed before commercial shipments and exports for food consumption. In this case, the seed are not viable because their embryos are damaged during the milling process. However, sometimes rice seed are transported without dehusking, including those that are intended for domestic seed sales. Also, viable seed can be dispersed when the grain is threshed and dried in the open air, and when it is handled, sorted, and transported for milling. A few studies have attempted to quantify the extent of gene flow by means of seed dispersal in rice or other cultivated species (Barton and Dracup 2000; Saji *et al.*, 2005). However, in the following sections, pollen-mediated gene flow from cultivated rice (*Oryza sativa*) to other rice crops, weedy rice (O. sativa f. *spontanea*), and wild rice species (other *Oryza* species) will be the main focus.
(Step four in Figure 4)

5.4. Expression and Inheritance of Transgene in Wild Relatives

There is a large amount of evidence demonstrating that genes can move from a crop to its wild or weedy populations *via* gene flow, the frequencies of which in some plant species can be relatively high (Ellstrand, 2003; Song *et al.*, 2003; Chen *et al.*, 2004; Wang *et al.*, 2006). This means that in many cases transgene escape from GM crops to their wild relatives is unavoidable. However, whether or not the escape of the transgene will have ecological or environmental consequences depends essentially on the extent to which the function of the transgene will be maintained (or changed) in wild relative receiving species. If the escaped transgene cannot express normally (i.e., with much lower levels of its products) in wild relative species following outflowing, the transgene may not alter the traits or fitness of the wild relatives. As a result, the transgene escape would not introduce any ecological consequences. On the other hand, if the transgene can express normally, or even stronger than in the parental GM crops, after its introgression into wild relative species, then the escaped transgene might provide a fitness advantage to the populations of recipient wild relatives, resulting in unwanted ecological consequences.

Therefore, to facilitate the biosafety assessment of transgene escape to populations of wild relative species, it is important to conduct scientific research to properly estimate the expression level of a particular transgene in wild individuals, as well as the inheritance of the transgene in wild populations under different environments. This is particularly relevant for transgenes that have obvious selective advantages for biotic (such as insect and disease) and abiotic (such as drought and salinity) stresses, if pollen-mediated transgene flow to wild relative species cannot be circumvented. In this case, questions relating to transgene expression and inheritance following introgression into the wild relatives are more important.

Currently, there are a number of methodologies to estimate transgene expression levels, including by **e**nzyme-**l**inked **i**mmuno**s**orbent **a**ssay (ELISA: Sims and Berberich, 1996; Bashirn *et al.*, 2005) and **r**everse **t**ranscriptase-**p**olymerase **c**hain **r**eaction (RT-PCR: Sripaoraya *et al.*, 2006). Commonly, the principle of estimating transgene expression is to measure the amount or level of transgene products (e.g. the *Bt* toxic protein) that can be detected in the individuals or populations of wild relatives, in comparison with the parental GM crops. For example, to determine whether an insect-resistance transgene (*Bt*) introgressed into wild populations will pose significant environmental consequences, under the hypothesis that the target lepidopteron species

occur in association with the wild crop relative population and regulate the population dynamics, estimations of transgenic expression levels in wild individuals will help predict the possibility of change to the wild population composition. Artificial hybrids and their progenies between a GM (e.g. *Bt*-insect resistance) crop and wild relatives can be produced by artificial crosses and backcrosses in order to estimate the transgene expression in wild species. The content of *Bt* protein can then be measured in the GM crop, F_1 hybrid and advanced-hybrid populations. If the content of *Bt* protein in the hybrid progenies of wild relatives is similar to the GM crop, then it is assumed that the transgene will be able to kill the target lepidopteran species in the new host wild population. On the other hand, if the content of *Bt* protein is dramatically low or undetectable in the hybrid progenies of wild relatives, it is assumed that the transgene will not cause further ecological consequences, due to "loss of function" in the wild populations.

Similarly, the inheritance of a transgene in the wild population can also be estimated by the production of artificial populations of F_1 hybrids and advanced progenies through crosses between a GM crop and wild relatives, and subsequent backcrosses and self-pollination. If the transgene is normally expressed in crop-wild hybrids and progenies, as well as inherited between different generations, further biosafety assessments of possible environmental consequences will be necessary.
(Step five in Figure 4).

5.5. Fitness Change of Wild Relatives Caused by Transgenes
For the estimation of long-term persistence and spread of transgenes in crop wild populations in relation to the fitness change, several key factors should be taken into consideration: 1) genetic mechanisms (e.g. genetic relationships and compatibility) that allow the transfer of transgenes into wild populations, 2) the degree (gene flow) to which transgenes are transferred to wild populations; 3) the fitness of early hybrids relative to their wild parents, and 4) possible fitness costs or benefits that are associated with a particular transgene (Jenczewski *et al.*, 2003).

If a transgene can move from a GM crop to its weedy or wild relatives, and at the same time the escaped transgene can be normally expressed and inherited in the wild relatives, it is then very important to continue the risk assessment in order to understand whether or not the transgene will change the ecological and evolutionary fitness of the recipient wild relatives. This

is because if expression of the transgene causes changes to the fitness of recipient wild relatives, the pattern of persistence and spread of the transgene in a wild population may vary significantly. Transgenes may have a strong expression pattern due to activation by a special promoter, and have very unique functions that may not be found in natural situations. Expressing a transgene may considerably alter the ability of wild relatives in terms of their survival, competition, and/or reproduction. These changes may affect the persistence and spread of transgene in wild populations in a spatial or temporal dimension. To establish crop-wild hybrid-and-progeny populations (e.g. producing F_1 hybrids, self-pollinated F_2, F_3 progenies, and backcrossed BC_1, BC_2 progenies) under experimental conditions through artificial crosses between a GM crop and its wild relatives will facilitate data generation for any fitness analysis required during the biosafety assessment.

However, to appropriately measure any fitness changes (sometimes the change can be very minor) of wild plants brought about by the expression of a transgene, and to properly incorporate the collected fitness data into the biosafety assessment system remains challenging. A well-designed fitness study can take a very long time to complete, especially when data may be required from multiple generations. It is important to point out that fitness is a measurement of the successful survival and reproduction of wild plants, which can be affected by many components throughout the life cycle in a given environment, e.g. seed dormancy and germination, seedling establishment and vegetative growth, individual viability and fecundity. Even though the determination of an adverse effect and the eventual success of a risk assessment largely relies on the prediction of any fitness change caused by transgenes, it is not straightforward to identify the components crucial for accurately predicting the fitness of recipient wild plants. Therefore, a few aspects need to be taken into consideration when studying fitness for the biosafety assessment.

The usual way to estimate any fitness change is to examine vegetative and reproductive productivity of crop-wild hybrids (mostly the early generations of hybrids), because morphological and reproductive traits appear to be more directly related to the number of offspring an individual can potentially produce (Arriola and Ellstrand, 1997; Snow *et al.*, 2003; Song *et al.*, 2004b; Hani *et al.*, 2005; Mercer *et al.*, 2007). However, the direct measurement of fitness change in F_1 hybrids poses some concerns on the correct judgment of a transgene in crop-wild hybrids because the role of lifetime fitness, competitive

advantage at the specific growth stages, and trade-offs between different components of fitness is still unclear (Jenczewski *et al.*, 2002). It is recognised that crop-wild hybrids will not be genetically uniform due to variation in wild populations (Linder and Schmitt, 1994), leading to difficulties in generalising the results from only studies of F_1 hybrids. According to the study by Linder *et al.* (1998), estimates of early generations of crop-wild hybrid fitness may be of little predictive value for the assessment of transgene establishment. Sometimes, the F_1 crop-wild hybrids demonstrate enhanced vegetative vigour that contributes to their total fitness, but this may not be of much use in predicting any ecological consequences over a long period of time.

It is also important to measure any long-term persistence and spread of crop genes (transgenes) in wild species after crop-wild hybridisation and introgression occurs (Campbell and Snow, 2007). The direct measures of crop gene establishment in early hybrids raise questions about distinguishing introgressive markers from ones jointly inherited from a common ancestor. Studies have therefore concentrated more on analysing the successive steps in the process of transgene establishment. Therefore, when designing a study to estimate any fitness change resulting from transgene flow, it is necessary to establish experimental populations of different generations of crop-wild hybrid progenies.

Evaluating the long-term spread and persistence of a transgene in wild populations also requires an understanding of whether possession of the transgene imposes a cost (fitness penalty) on the wild plants in the absence of a selection pressure on the transgene. It is also important to know whether the transgene confers a trait with selective advantage or if it is selectively neutral. Transgenes with different selective values (advantageous, neutral, or disadvantageous) are expected to spread in wild populations at quite different rates. In addition, apart from the balance between the fitness costs and benefits of a transgene brought to wild plants under natural selection, the persistence and spread of a transgene in wild populations will depend on the strength and frequency of transgene flow. Strong and recurrent transgene flow can be sufficient to establish transgenes in populations, even though the transgenes may contribute to slight fitness disadvantages. The frequency of transgenes can be expected to increase in the wild populations if crop-to-wild transgene flow is significantly strong and frequent. Taken together, all these factors will significantly affect the dynamics of a wild population that has acquired a transgene(s) through pollen-mediated gene flow, significantly

impacting the risk assessment procedure.
(Steps six and seven in Figure 4).

When all the steps of the risk assessment procedure (Figure 4) are completed, via published literature consultation and data collection from actually designed experiments, it should be possible to make a conclusion with a high degree of confidence concerning any environmental consequences caused by the transgene outflow to a crop wild relative species. The risk assessment exercise not only provides us with a tool to determine the possibility of transgene escape to the wild relatives, but also allows the appropriate measurement of adverse affects caused by a particular transgene that has been incorporated into a population of wild relatives. This will help facilitate decision-making concerning an application for environmental release and commercial production of a GM crop in a particular region under unique environmental conditions.

6. MANAGEMENT AND MITIGATION OF POLLEN-MEDIATED TRANSGENE ESCAPE

As shown in the above analyses and discussion, pollen-mediated gene flow can effectively contribute to the extent of transgene escape from a GM crop to its non-GM counterparts and wild relatives. Therefore, effective confinement or even elimination of pollen-mediated gene flow is an important measure for the management of transgene escape. There are two major groups of strategies being proposed to confine pollen-mediated gene flow: physical and biological. However, it is important to point out that the confinement of transgene flow is extremely difficult in many cases, particularly for crop-to-crop gene flow in plant out-breeding species and crop-to-wild gene flow when crops and their wild relatives co-exist or occur in close proximity.

6.1. Physical Confinement

In many cases, it is possible to significantly reduce the frequency of pollen-mediated gene flow by deploying an effective strategy of physical isolation between GM pollen donors and recipients. The extent of pollen-mediated gene flow is affected by pollen flow that has a leptokurtic distribution, with most pollen grains spreading close to the pollen donors, and only a small amount moving over longer distances. For example, most maize pollen falls within about 30 m, and most rice pollen falls within a few metres, from the pollen donor (Song et al., 2004a; Devos et al., 2005). Again, the longevity of

pollen viability for many crop species is only a matter of minutes. For example, the imperial expectation of rice pollen longevity is less than 10 minutes, although the pollen viability of wild rice and its hybrids can be somewhat longer (Song *et al.*, 2001). Temporal (flowering time) isolation between pollen donors and recipients can sometimes also serve as an effective strategy for physical confinement.

Frequencies of pollen-mediated gene flow can vary significantly among plant species, even among different varieties of the same crop species (Rong *et al.*, 2004). Many experimental and modelling studies have been conducted with different crop species for identifying a useful measure of the ideal physical isolation between GM and non-GM crops to contain "contamination" (also referred to as "adventitious presence") under an acceptable agreed level, for example, in rice (Song *et al.*, 2004a; Rong *et al.*, 2007), maize (Luna *et al.*, 2001; Devos *et al.*, 2005), and wheat (Gustafson, *et al.*, 2005; Hansona *et al.*, 2005). The objective of physical isolation is to maintain the level of gene flow below certain set thresholds. There will never be a standardised physical isolation strategy for all crop species, and the effectiveness of a strategy must be measured on a crop-by-crop and crop-to-wild basis.

6.1.1. Spatial isolation

Spatial isolation involves a separation zone that could be open land or fields with other plant species that serve as a pollen barrier between the GM pollen donors and recipients. Gene flow mediated by pollination should be within the range of pollen flow of a particular species, and the frequency of pollen-mediated gene flow is determined by the pollen density around the pollen recipient at particular spatial distances. This suggests that spatial isolation can reduce gene flow between GM and non-GM crops. A pollen barrier that is a band of plants grown around pollen donors and/or recipients allows conventional crops to be isolated from their GM crop counterparts by distance. Pollen barrier space may be more effective than other forms of physical separation (Devos *et al.*, 2006). Pollen barriers, for example pollen-producing (competition with GM pollen) or tall crops, hedges, trees, and screens are usually recommended around recipient fields. This strategy is based on studies showing that cross-fertilisation (gene flow) rates are usually higher at crop edge rows than closer to the centre of fields. However, the merits of pollen barriers in different crop configurations are still uncertain. It was shown that the use of large trap crops reduced long-distance pollen flow significantly, but sometimes the use of such large trap crops is unrealistic in practice because the borders of trap crops can be larger

than the transgenic fields (Hokanson *et al.*, 1997). Changes in environmental conditions, in particular due to metrological variation, will strongly affect the effectiveness of spatial isolation between GM and non-GM crops. For example, the change of wind strength and speed can affect the effectiveness of pollen barriers, because pollen can be carried to high altitudes by the wind current and land on plants far from the donors (Devos *et al.*, 2005).

6.1.2. Temporal isolation
The idea of temporal isolation is to separate the flowering time of GM from non-GM crops or totally remove pollen from the GM crops. Temporal strategies involve the use of delayed plantings and crop rotation to avoid contact between GM and non-GM crops. In practice, staggering the sowing times of different crops may help to reduce gene flow by changing their flowering times. If crops do not have overlapping flowering periods, the chances of hybridisation are greatly reduced. A study in Spain showed a significant reduction in cross-fertilisation by sowing crops no more than one week apart. There was a reduction of 75 % in gene flow when the time difference was stretched to three weeks (reported in Devos *et al.*, 2005). It is recommended that crop rotation could be useful to minimise contact with non-GM crops if neighbouring farmers are able to closely coordinate with their crop(s), but this may not be possible in places where mono-cropping is widespread (Devos *et al.*, 2005). Apart from the above constraints, another difficulty of this strategy is the market price for the harvested crops, because the earlier crop products usually have a better market price. This approach may also be undesirable for crops in some environments. For example, maize may be damaged by frost if planted early, and delaying sowing may compromise crop yield (Devos *et al.*, 2005). For photoperiod-sensitive crops, it may also be difficult to separate flowering of different plants by sowing crops at short-time differences. In addition, removal of the pollen-producing tassels from maize plants (de-tasseling) is an effective way to breed maize varieties without allowing gene escape. It is usually done by hand due to the variation in plant height, but can also be mechanised. However, it is only feasible for small plots (Gurian-Sherman, 2006) and would be difficult to maintain in commercial agriculture (Luna *et al.*, 2001).

6.1.3. GM crop-free zones
The proper deployment of GM and non-GM crops in a region or in a country can be an effective strategy to totally avoid pollen-mediated transgene flow and GM contamination/adventitious mixing. For example, growing GM cotton or GM rice in a region where no wild relatives are found will avoid transgene

escape to wild relatives through pollen-mediated gene flow. A strategic deployment of a GM crop-free zone and its implementation needs efforts and inputs from regional authorities and governments. If such a strategy is to be designed and implemented, then a robust ordination and legislation system should be established for its management. It may be possible for farmers to declare their region a GM crop-free zone or GM crop production zone on the basis of voluntary agreements, possibly supported by governments. This may be the most effective and least costly measure to ensure the physical confinement of GM crops and to promote co-existence on a regional basis (Devos *et al.*, 2005).

6.1.4. Other concerns for physical confinement
The success of physical confinement of transgene(s) from gene flow is also dependant upon the ability to cultivate crops in fields over time. Fields used for cultivating GM crops may not be suitable for cultivating non-GM crops in immediately following years due to volunteer growth. Volunteers and stray seed containing transgenic traits within and around fields may hybridise with other related non-GM crops and wild relatives. Therefore, confinement of transgenes depends on the proper disposal of plants and the seed-bank after harvest by removing volunteers and stray seed. It is unrealistic to expect that all GM volunteer growth from all kinds of crops could be prevented. In fact, most cases of contamination/adventitious mixing with non-GM crops are likely to be associated with volunteers and stray seeds. Studies can measure the likelihood of persistent GM volunteer populations growing within or near non-GM fields and whether it would result in enough cross-pollination to push GM adventitious presence over agreed thresholds (Flannery *et al.*, 2005).

6.2. Biological Confinement
Confinement of pollen-mediated transgene flow can be accomplished not only by physical means, but also by biological means. Confinement based on the biological nature of organisms can be used to prevent cross-pollination of GM and non-GM crops, although this is still largely under development. Biological confinement considers how biological and genetic engineering techniques, such as induced sterility and engineering plants not to produce pollen, can prevent GM plants from escaping into natural ecosystems and breeding or competing with their wild or weedy relatives, or passing genetically modified traits to other species. Modern biotechnology makes the new confinement strategy possible. Biological confinement offers a strategy to design a "cleaner" GM product through appropriate planning

and design - whether and how to confine a GM crop - before the production of a GM plant.

A variety of biological confinement strategies have been devised to augment or replace physical containment strategies (Gressel, 2000; Daniell, 2002; Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Genetic barriers that could be implemented to minimise gene flow between GM and non-GM crops include chloroplast transformation, male sterility, apomixis, and ploidy level (Box 5). The efficacy of biological confinement methods will vary depending on the GM crops and the environment in which the GM crops will be released. Transgene confinement will be more effective over short time scales and small geographic areas. It is important to point out that, in most cases, a single biological confinement method is unlikely to achieve complete confinement, and therefore, it is recommended that the development of GM crops include more than one confinement method to lower the chance of failure.

BOX 5. TYPES OF BIOLOGICAL CONFINEMENT

Cloroplast Transformation – The insertion and expression of a transgene(s) in plant chloroplasts, mediated usually by particle bombardment or direct DNA uptake into protoplasts. Unlike nuclear transformation, has the benefit of maternal inheritance in most crop species, remarkably high expression levels, and ability to process polycistronic mRNA.

Male sterility – The condition in which the male gametes are either absent, deficient in number, or non-functional. For example, in plants, male sterility indicates a situation where pollen grains of an individual are completely or partially aborted.

Apomixes – Any kind of reproduction without fertilisation or fusion between male (sperm cells) and female gametes (egg cells). As a result, apomictically produced seeds are genetically identical to the parental plants.

Cleistogamy – A type of trait in certain plant species to produce seeds by using non-opening and self-pollinating flowers. This behaviour is most widespread in legumes, for example in peanuts, peas, and beans.

Further research to understand how well specific methods work, and well planned combinations of confinement methods, will need to be tested in organisms with representative genetic profiles and in a wide variety of field environments. Research aimed at developing new biological confinement methods will further minimise risks and may help boost public confidence in modern biotechnology.

There are many approaches being proposed or developed for the biological confinement of transgene flow from GM crops (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Some of the approaches are based on pre-existing agronomic or horticultural methods, others are newly developed, and some are hypothetical. These approaches are designed according to their purposes of confinement, for example:
  1) confining all gene flow via pollen and seeds;
  2) reducing the spread and persistence of vegetative propagules;
  3) confining pollen only;
  4) confining transgenic traits only;
  5) reducing gene flow to and from wild relatives;
  6) using phenotypic and fitness handicaps to reduce the need for confinement; and
  7) reducing the exposure to transgenic products in plants.

The major approaches for confining pollen-mediated transgene flow that are being commonly discussed in the scientific literature are introduced below.

6.2.1. Chloroplast transformation
The genetic modification of chloroplasts is a potentially powerful technology that has served as a mode for the biological confinement of transgene outflow through pollen grains (Box 5).

This technology was first developed for tobacco (Svab *et al*., 1990) and was later extended to many other plant species (e.g., Daniell *et al*., 1998; Khan and Maliga, 1999). The pollen of most major crop plants does not contain plastids, and chloroplast genes can only be transmitted through the egg to the embryo. As such, the risk of pollen-mediated transgene escape will be rare, but not totally eliminated, if the transgene of interest is inserted into the chloroplast genome (Scott and Wilkinson, 1999). Chloroplast transformation can dramatically reduce the likelihood of pollen-mediated transgene outflow because of the maternal inheritance of chloroplasts in most angiosperms (Birky,

1995). Therefore, this confinement strategy is considered as a promising tool in biotechnology that has the potential to solve the problems of transgene escape, not only to wild or weedy relatives, but also to the non-GM counterparts. In addition, chloroplast transformation has the benefit of remarkably high levels of stable transgene expression (McBride *et al.*, 1995; Kooter *et al.*, 1999; Ye *et al.*, 2000). Chloroplasts also have the capacity to express multiple genes from a polycistronic mRNA, which allows the pyramiding of genes to, for example, decrease the risk of promoting resistance in pest organisms (Gressel, 1999).

However, it is argued that pollen-mediated gene flow is two-directional, meaning that while GM crops disseminate their pollen, at the same time, they also receive pollen from other plants, including their wild relative species. This reciprocal process could also result in hybridisation between GM crops and wild relatives (Lu, 2003). Hybrids with transgenic crops as the maternal parent and wild relatives as the paternal parent will carry transgenes. These hybrids and their backcrossed progenies with the wild parents could become potential weeds in agricultural systems. In addition, other concerns of using this technology are whether plasmid DNA can be inherited paternally (via pollen). In fact, approximately one-third of the flowering plants investigated exhibited some degree of paternal or bi-parental plasmid inheritance, and paternal transmission of chloroplasts does occur rarely in some species (Mogensen and Rusche, 2000; Huang et al., 2003). Chloroplast genes can also sometimes move into the nuclear genome, although at an extremely low frequency (6×10^{-5}; Huang *et al.*, 2003). These highlight the weakness of applying only this strategy to confine pollen-mediated transgene flow. More research is needed in this promising area to perfect this approach for biological confinement of transgenes.

6.2.2. Male sterility
Male sterility (Box 5) is used as the main control mechanism in conventional hybrid breeding for seed production as self-pollination is prevented. It has been identified in many crops, including rice, maize, wheat, brassicas, alfalfa, rose clover, birds-foot trefoil, carrot, and onions (Kaul, 1988), but experiences with various crop plants shows that male sterility is nearly never perfect. For example, the development of hybrid alfalfa varieties has not been commercially viable due to the reduced pollination of male-sterile rows by bees. The deployment of male sterile crops developed by transgenic methods could be very useful for transgene confinement because it can greatly reduce pollen-mediated crop-to-crop and crop-to-wild transgene flow, particularly in perennial

forage crops where the potential for gene transfer is high. In addition, male sterility can also be useful for the deployment of transgenic open-pollinated varieties harvested for vegetative organs. Transgenic male sterility could be introduced to crops for which natural genic or cytoplasmic systems do not exist. However, most types of male sterility are imperfect, meaning there is always a certain percentage of fertile pollen grains present, particularly under different environmental conditions. Also, the transgenic method could fail if gene silencing or recombination separates the confined gene from the sterility system (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Therefore, the use of male sterility alone to confine pollen-mediated transgene flow has disadvantages.

6.2.3. Apomixes

Apomixes (Box 5) describes the production of seed through an asexual process, and occurs naturally in some plant species (e.g., Grant, 1981; Wang *et al.*, 1993). In apomixis, as in vegetative propagation, daughter plants are genetically identical to mother plants, and uniform within and between generations. The introduction of apomixis into crops traditionally propagated through seeds could facilitate the fixation and propagation of superior hybrid genotypes to the final products. Because obligatory apomictic plants do not require the fusion of their own male and female gametes to produce progenies and cannot be fertilised by gametes from other plants to produce progenies, apomixis has been suggested as a tool for biological confinement of pollen-mediated gene flow (Gressel, 1999; Daniell, 2002). If it is possible to produce a GM crop that is fully asexual and fully male-sterile, for example in some varieties of potato, then pollen-mediated gene flow can nearly never happen because there is no possibility for hybridisation with nearby non-GM crops or wild relatives. However, the commercial application of apomixis in major crops will still take many years to develop, if it is possible at all, because obligate apomixis is extremely rare and moderate to high pollen fertility is common in apomictic plants in nature. In addition, due to the population genetics of an apomictic allele linked to the transgene (e.g. sweep effect), it is necessary to make sure that apomictic GM plants will not become invasive.

6.2.4. Cleistogamy and ploidy level

The flowers of cleistogamous plants (Box 5) either never open, or eventually open only after fertilisation has been completed (e.g. in some varieties of cultivated barley). These plants are strictly self-fertilised with limited amounts of pollen. Theoretically, obligate cleistogams would not be able to fertilise

117

other plants, nor able to be fertilised by other plants. Therefore, creating plants with obligate cleistogamy is recommended as a possible approach for biological confinement of pollen-mediated transgene outflow (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Biological confinement of transgenes by the use of cleistogamy seems to be possible for some crop species, but it is not practical for all crop species. Many crops, such as maize, common buckwheat, cassava, and most cucurbits, are allogamous or cross-fertilised, and it is difficult to create cleistogamous plants for such crops (Lu, 2003). Therefore, the application of such an approach to confine transgene flow will be applicable to only certain crops.

Many open-pollinated species are polyploids in nature, for example common wheat is hexaploid (2n=6x=42), durum wheat is tetraploid (2n=4x=28), and perennial wheatgrass has both diploid (2n=2x=14) and tetraploid (2n=4x=28) cultivars (Lu, 1993). Deploying transgenes at different ploidy levels is a potential genetic barrier for transgenes to transfer to wild populations because hybrids between different ploidy levels are commonly sterile. For example, gene flow from hexaploid (2n=6x=48) white clover to wild tetraploid populations of white clover would be severely reduced or eliminated. Another strategy of using polyploidy is to deploy transgenes at different ploidy levels with incompatible genomes. This is based on the fact that many cultivated crops have multiple genomes and for a particular crop, only one genome is in principle compatible for interspecific hybridisation with its wild relatives. In other words, the extent of transgene exchange from an allopolyploid crop to its wild diploid relatives depends on the genome where the transgene is located. Gene introgression between different genomes with low homology tends to be low. Therefore, the risk of transgenes spreading into wild relatives can be significantly reduced in this system. However, this approach may not be possible for all crops, because incompatibility of genomes is not strict. Cytological studies show a considerable amount of genetic recombination between different (incompatible) genomes, particularly when genetically-controlled promoters for chromosome pairing are involved (Sears, 1983; Lu, 1993). In addition, some hybrids between crop species and their wild relatives are perennial, and even if no immediate introgression occurs between the genomes of a GM crop and its wild relatives, transgenes may subsist in the hybrids that can propagate vegetatively without producing seeds.

118

There are a number of other molecular approaches being proposed for GM crop transgene confinement, such as seed sterility that involves genetic use

restriction technologies (GURTs), excision of transgene before reproduction, and repressible lethal seed confinement, and have been described elsewhere in detail (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

6.3. Transgene Mitigation

It is well recognised that confining transgene flow from a GM crop to wild relatives is not always desirable and straightforward, and is dependant upon the choice of approach (e.g. physical or biological confinement) adopted in the actual confinement practices. Although there are a number of confinement methods that have been developed or proposed for the objective of minimising the flow of transgenes into wild relatives, a certain level of transgene flow (leakage) is always inevitable. This is particularly true for such crop species as rice and sunflowers that have conspecific weeds, as well as for those that have closely-related weedy species, such as oilseed rape, sorghum, barley, and maize. Given the fact that the confinement of pollen-mediated crop-to-wild gene flow is nearly impossible in reality, a strategy to mitigate the impact of transgene escape if the escape is inevitable has been proposed (Gressel *et al.*, 1999; Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

Transgenic mitigation (TM) has received growing attention as an approach for confining transgene spread in wild populations by compromising the fitness of weeds that receive positive survival traits from crop genes through introgression (Gressel, 2000, 2002; Hani *et al.*, 2004, 2006). In this concept, so-called "mitigator" genes are introduced into a GM crop and tandemly-linked to the primary desired transgene(s). The "mitigator" genes would specifically reduce the fitness of any hybrids and their progenies resulting from pollen-mediated transgene flow, considerably reducing any negative environmental consequences. According to Gressel (1999, 2002), the transgenic-mitigation (TM) approach is based on the premises that: 1) tandem constructs act as tightly-linked genes with exceedingly rare segregation from each other; 2) the TM traits chosen are neutral or favourable to crops, but deleterious to non-crop progeny; and 3) individuals bearing even mildly harmful TM traits will remain at very low frequencies in weed/wild populations because weeds typically have a very high seed output and strongly compete among themselves, eliminating even marginally unfit individuals. Therefore, if the target transgene providing the agricultural advantage is flanked in a tandem construct by mitigator genes such as dwarfing, uniform seed ripening, non-

119

shattering, anti-secondary dormancy, or non-bolting genes, the overall effect would be deleterious after introgression into wild or weedy relatives as the TM genes will reduce the competitive ability of the transgenic hybrids. As a consequence, these hybrids will poorly compete with normal wild plants, and therefore the transgenes will persist in only low frequencies in agricultural ecosystems. Successful attempts to apply TM technology in tobacco have been reported (Al-Ahmad et al., 2006).

There are, however, still some concerns over the use of transgenic mitigation. For example, the technology can not solve the problems of massive amounts of transgenes moving into weedy or wild species through recurrent gene flow. The destiny and long-term consequences of the mitigator genes in crop and weedy or wild populations are unpredictable (Lu, 2003). In addition, establishing tandem constructs with tightly-linked genes will require considerable efforts for multi-gene engineering. Future attempts to transfer multiple genes with different traits into one crop variety may challenge such constructs particularly. The long-term ecological consequences resulting from these transgenes as a whole package in the environment is as yet unknown and will be difficult to predict.

Although still being discussed and argued, transgenic mitigation technology brings new insight for effective management of transgene flow and its environmental consequences by mitigating the risks to a minimum level. Probably, there is no single approach that can be very effective to confine transgene escape to wild relatives and to mitigate the consequences from such an escape. Also, it is not necessary to put the same effort to confine all the transgenes from all GM crops under all environmental conditions where GM crops will be released for cultivation. This is because many transgenes that do not provide a selective advantage to the host plant in nature may not pose any environmental consequences, and many crops that have extremely low gene flow frequencies (e.g. some legume species) already have a low risk of transgene escape. In addition, for some geographical locations where wild relatives or conspecific weeds of the GM crops are absent, transgene escape through pollen-mediated gene flow would not be an issue. A strategic combination of transgene confinement from gene flow and mitigation to minimise its impacts in a particular circumstance should provide an effective strategy to manage any environmental consequences caused by transgene escape.

7. CONCLUSION

Gene flow *per se* is not a risk because it is a natural process and a part of evolution that happens incessantly and permanently. Transgene escape from a GM crop to its non-GM crop counterparts and populations of wild or weedy relatives through gene flow may pose potential biosafety problems for food and health, environment, and socio-economics and ethics. Potential environmental consequences from transgene escape essentially depends on whether or not the transgenes will express normally in wild relatives, and whether or not the transgenes will change the fitness of introgressed plants, which will determine the dynamics of populations that have maintained the transgene under a selection pressure. Based on biological knowledge, the possibility of transgene escape can be assessed effectively, as well as the potential environmental consequences created by any transgene outflow. It is possible to significantly reduce transgene outflow by the use of a proper combination of confinement strategies and methodologies, applying both physical and biological means as use of any one method in isolation is likely to be ineffective. It is also possible to reduce the impacts of a transgene in the environment even further by the use of transgenic mitigation methods if confinement ultimately fails. Strategies or methodologies for transgene flow containment and mitigation are new and very little testing has been done to verify their long-term effectiveness. However, the availability of these strategies offers an opportunity for the future management of transgene escape to wild relatives and thereby further minimising any related environmental consequences.

8. ACKNOWLEDGEMENTS

9. REFERENCES

Aerni P 2007. Agricultural biotechnology and its contribution to the global knowledge economy. In: *Green Gene Technology. Research in an Area of Social Conflict*, Fiechter A & Sautter C (eds). Series: *Advances in Biochemical Engineering/ Biotechnology* 107: 69-96. DOI: 10.1007/10_2007_058

Ahmed FE 2002. Detection of genetically modified organisms in foods. *TRENDS in Biotechnology* 20(5): 215-223. DOI: 10.1016/S0167-7799(01)01920-5

Al-Ahmad H, Dwyer J, Moloney M and Gressel J 2006. Mitigation of establishment of *Brassica napus* transgenes in volunteers using a tandem construct containing a selectively unfit gene. *Plant Biotechnology Journal* 4: 7–21. DOI: 10.1111/j.1467-7652.2005.00152.x

Andow DA and **Zwahlen** C 2006. Assessing environmental risks of transgenic plants. *Ecology Letters* 9: 196–214. DOI: 10.1111/j.1461-0248.2005.00846.x

Arriola PE and **Ellstrand** NC 1997. Fitness of interspecific hybrids in the genus sorghum: persistence of crop genes in wild populations. *Ecological Applications* 7(2): 512–518. DOI: 10.1890/1051-0761(1997)007[0512:FOIHIT]2.0.CO;2

Aumaitre A, **Aulrich** K, **Chesson** A, **Flachowsky** G and **Piva** G 2002. New feeds from genetically modified plants: substantial equivalence, nutritional equivalence, digestibility, and safety for animals and the food chain. *Livestock Science* 74: 223–238. DOI: 10.1016/S0301-6226(02)00016-7

Baack EJ, **Sapir** Y, **Chapman** MA, **Burke** JM, **Rieseberg** LH 2008. Selection on domestication traits and quantitative trait loci in crop-wild sunflower hybrids. *Molecular Ecology* 17(2): 666–677. DOI: 10.1111/j.1365-294X.2007.03596.x

Bahieldin A, **Mahfouz** HT, **Eissa** HF, **Saleh** OM, **Ramadan** AM, **Ahmed** IA, **Dyer** WE, **El-Itriby** HA and **Madkour** MA 2005. Field evaluation of transgenic wheat plants stably expressing the *HVA1* gene for drought tolerance. *Physiologia Plantarum* 123: 421–427. DOI: 10.1111/j.1399-3054.2005.00470.x

Barton JE and **Dracup** M 2000. Genetically modified crops and the environment. *Agronomy Journal* 92: 797-803. http://agron.scijournals.org/cgi/reprint/92/4/797

Bashirn K, **Husnain** T, **Fatima** T, **Riaz** N, **Makhdoom** R and **Riazuddin** S 2005. Novel indica basmati line (B-370) expressing two unrelated genes of *Bacillus thuringiensis* is highly resistant to two lepidopteran insects in the field. *Crop Protection* 24: 870–879. DOI: 10.1016/j.cropro.2005.01.008.

Bates SL, **Zhao** JZ, **Roush** RT and **Shelton** AM 2005. Insect resistance management in GM crops: Past, present and future. *Nature Biotechnology* 23: 57–62. DOI: 10.1038/nbt1056

Bellon MR and **Berthaud** J 2004. Transgenic maize and the evolution of landrace diversity in Mexico: The importance of farmers' behavior. *Plant Physiology* 134: 883–888. http://www.plantphysiol.org/cgi/reprint/134/3/883

Birch ANE, **Griffiths** BS, **Caul** S, **Thompson** J, **Heckmann** LH, **Krogh** PH and **Cortet** J 2007. The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: A review of the ECOGEN project. *Pedobiologia* 51: 251–260. DOI: 10.1016/j.pedobi.2007.04.008

Birky CW 1995. Uniparental inheritance of mitochondrial and chloroplast genes: Mechanisms and evolution. *Proceedings of the National Academy of Sciences USA*, 92: 11331-11338. http://www.pnas.org/cgi/reprint/92/25/11331

Bock R 2007. Plastid biotechnology: prospects for herbicide and insect resistance, metabolic engineering and molecular farming. *Current Opinion in Biotechnology* 18: 100–106. DOI: 10.1016/j.copbio.2006.12.001

Campbell LG and Snow AA 2007. Competition alters life history and increases the relative fecundity of crop–wild radish hybrids (*Raphanus* spp.). *New Phytologist* 173: 648–660. DOI: 10.1111/j.1469-8137.2006.01941.x

Cao QJ, **Lu** B-R, **Xia** H, **Rong** J, **Sala** F, **Spada** A and **Grassi** F 2006. Genetic

123

diversity and origin of weedy rice (*Oryza sativa* f. *spontanea*) populations found in Northeastern China revealed by simple sequence repeat (SSR) markers. *Annals of Botany* 98: 1241–1252. DOI: 10.1093/aob/mcl210

Celis C, **Scurrah** M, **Cowgill** S, **Chumbiauca** S, **Green** J, **Franco** J, **Main** G, **Kiezebrink** D, **Visser** RGF and **Atkinson** HJ 2004. Environmental biosafety and transgenic potato in a centre of diversity for this crop. *Nature* 432: 222–225. DOI: 10.1038/nature03048

Chamberlain D and **Stewart** NC 1999. Transgene escape and transplastomics. *Nature Biotechnology* 17: 330–331. DOI: 10.1038/7882

Chen G, **Wang** Z, **Liu** QQ, **Xiong** F, **Gu** YJ and **Gu** GJ 2006. Development and substance accumulation of caryopsis in transgenic rice with antisense *Wx* gene. *Rice Science*, 13: 106–112.

Chen LJ, **Lee** DS, **Song** ZP, **Suh** HS and **Lu** B-R 2004. Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. *Annals of Botany* 93: 67-73. http://aob.oxfordjournals.org/cgi/reprint/93/1/67

Christou P 1997. Rice transformation: Bombardment. *Plant Molecular Biology* 35: 197–203.

Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants 2002. *Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation. National Research Council* (NRC), National Academies Press, Washington DC, USA. http://www.nap.edu/openbook.php?isbn=0309082633

Committee on Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment 1989. *Field Testing Genetically Modified Organisms: Framework for Decision.* National Research Council (NRC), National Academy Press, Washington DC, USA. http://www.nap.edu/catalog.php?record_id=1431

Committee on the Biological Confinement of Genetically Engineered Organisms 2004. *Biological Confinement of Genetically Engineered Organisms.* National Research Council (NRC), National Academies Press, Washington DC, USA. http://www.nap.edu/openbook.php?isbn=0309090857

Conner AJ, **Glare** TR and **Nap** JP 2003. The release of genetically modified crops into the environment - Part II. Overview of ecological risk assessment. *Plant Journal* 33: 19–46. DOI: 10.1046/j.0960-7412.2002.001607.x

Cromwell GL, **Henry** BJ, **Scott** AL, **Gerngross** MF, **Dusek** DL and **Fletcher** DW 2005. Glufosinate herbicide-tolerant (LibertyLink®) rice vs. conventional rice in diets for growing-finishing swine. *Journal of Animal Science* 83: 1068–1074. http://jas.fass.org/cgi/reprint/83/5/1068

Curtis KR and **Moeltner** K 2007. The effect of consumer risk perceptions on the propensity to purchase genetically modified foods in Romania. *Agribusiness* 23: 263–278. DOI: 10.1002/agr.20116

Dale PJ, **Clarke** B and **Fontes** EMG 2002. Potential for the environmental impact of transgenic crops. *Nature Biotechnology* 20: 567–574. DOI: 10.1038/nbt0602-567

Dalecky A, **Bourguet** D and **Ponsard** S 2007. Does the European corn borer disperse enough for a sustainable control of resistance to *Bt* maize via the High Dose/Refuge strategy? *Cahiers d'Etudes et de Recherches Francophones / Agricultures* 16: 171–176. DOI : 10.1684/agr.2007.0097

Daniell, H 2002. Molecular strategies for gene containment in transgenic crops. *Nature Biotechnology* 20: 581–586. DOI: 10.1038/nbt0602-581

Daniell H, **Datta** R, **Varma** S, **Gray** S and **Lee** SB 1998. Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nature Biotechnology*, 16: 345–348. DOI: 10.1038/nbt0498-345

Daniels R, **Boffey** C, **Mogg** R, **Bond** J and **Clarke** R 2005. *The Potential for Dispersal of Herbicide Tolerance Genes from Genetically-Modified, Herbicide-Tolerant Oilseed Rape Crops to Wild Relatives*. Contract reference EPG 1/5/151, DEFRA, UK. http://www.defra.gov.uk/environment/gm/research/pdf/epg_1-5-151.pdf

Datta K, **Baisakh** N, **Thet** KM, **Tu** J and **Datta** SK 2002. Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. *Theoretical and Applied Genetics* 106: 1–8. DOI: 10.1007/s00122-002-1014-1

Datta K, **Vasquez** A, **Tu** J, **Torrizo** L, **Alam** MF, **Oliva** N, **Abrigo** E, **Khush** GS and **Datta** SK 1998. Constitutive and tissue-specific differential expression of the *cryIA(b)* gene in transgenic rice plants conferring resistance to rice insect pest. *Theoretical and Applied Genetics* 97: 20–30. DOI: 10.1007/s001220050862

Devos Y, **Reheul** D and **Schrijver** AD 2005. The co-existence between transgenic and non-transgenic maize in the European Union: A focus on pollen flow and cross-fertilization. *Environmental Biosafety Research* 4: 71–87. DOI: 10.1051/ebr:2007005

Devos Y, **Reheul** D and **Schrijver** AD 2006. Considerations of cross-fertilization between GM and non-GM maize. *ISB News Report* March 2006. http://www.isb.vt.edu/articles/mar0603.htm

Einsele A 2007. The gap between science and perception: The case of plant biotechnology in Europe. In: *Green Gene Technology. Research in an Area of Social Conflict*, Fiechter A & Sautter C (eds). Series: *Advances in Biochemical Engineering/ Biotechnology* 107: 1–11. DOI: 10.1007/10_2007_055

Ellstrand NC 2001. When transgenes wander, should we worry? *Plant Physiology* 125: 1543-1545. http://www.plantphysiol.org/cgi/reprint/125/4/1543

Ellstrand NC 2003. Current knowledge of gene flow in plants: Implications for transgene flow. *Philosophical Transactions of the Royal Society B: Biological Sciences* 358(1434): 1163–1170. DOI: 10.1098/rstb.2003.1299

Ellstrand NC and **Elam** D 1993. Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology Evolution and Systematics* 24: 217–242. doi:10.1146/annurev.es.24.110193.001245

Ellstrand NC, **Garner** LC, **Hegde** S, **Guadagnuolo** R, **Blancas** L 2007. Spontaneous hybridization between maize and teosinte. *Journal of Heredity* 98: 183–187. DOI: 10.1093/jhered/esm002

Ellstrand NC, **Prentice** HC and **Hancock** J.F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539–563. DOI: 10.1146/annurev.ecolsys.30.1.539

Engels JMM, **Ebert** AW, **Thormann** I and **De Vicente** MC 2006. Centres of crop diversity and/or origin, genetically modified crops and implications for plant genetic resources conservation. *Genetic Resources and Crop Evolution* 53: 1675–1688. DOI: 10.1007/s10722-005-1215-y

Ervin DE, **Welsh** R, **Batie** SS and **Carpentier** CL 2003. Towards an ecological systems approach in public research for environmental regulation of transgenic crops. *Agriculture Ecosystems and Environment* 99: 1–14. DOI: 10.1016/S0167-8809(03)00145-2

Finucane ML and **Holup** JL 2005. Psychosocial and cultural factors affecting the perceived risk of genetically modified food: An overview of the literature. *Social Science and Medicine* 60: 1603–1612. DOI: 10.1016/j.socscimed.2004.08.007

Fitt GP, **Andow** DA, **Carriere** Y, **Moar** WJ, **Schuler** TH, **Omoto** C, **Kanya** J, **Okech** MA, **Arama** P and **Maniania** NK 2004. Resistance risks and management associated with *Bt* maize in Kenya. In: *A Case Study of Bt Maize in Kenya*, Hilbeck A and Andow DA (eds.), CAB International, Wallingford, UK. pp. 209–250.

Flannery ML, **Meade** C and **Mullins** E 2005. Employing a composite gene-flow index to numerically quantify a crop's potential for gene flow: An Irish perspective. *Environmental Biosafety Research* 4: 29-43. DOI: 10.1051/ebr:2005009

GalloMeagher M and **Irvine** JE 1996. Herbicide resistant transgenic sugarcane plants containing the bar gene. *Crop Science* 36 (5): 1367–1374

Gealy DR, **Mitten** DH and **Rutger** JN 2003. Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*): Implications for weed management. *Weed Technology* 17: 627–645. DOI: DOI: 10.1614/0890-037X(2003)017[0627:GFBRRO]2.0.CO;2

Gepts P and **Papa** R 2003. Possible effects of (trans)gene flow from crops on the genetic diversity from landraces and wild relatives. *Environmental Biosafety Research* 2(2): 89–103. DOI: 10.1051/ebr:2003009

Giovannetti M, **Sbrana** C and **Turrini** A 2005. The impact of genetically modified crops on soil microbial communities. *Rivista Di Biologia-Biology Forum* 98: 393–417.

Grant V 1981. *Plant Speciation*. 2nd Edition. Columbia University Press, New York, USA.

Gressel J 1999. Tandem constructs: Preventing the rise of superweeds. *TRENDS in Biotechnology* 17: 361–366. DOI: 10.1016/S0167-7799(99)01340-2.

Gressel J 2000. Molecular biology of weed control. *Transgenic Research* 9(4-5): 355–382. DOI: 10.1023/A:1008946628406

Gressel J 2002. *Molecular biology of weed control.* Taylor & Francis, London, UK.

Gura T 1999. Biotechnology: New genes boost rice nutrients. *Science* 285(5430): 994-995. DOI: 10.1126/science.285.5430.994

Gurian-Sherman D 2006. *Contaminating the Wild? Gene Flow from Experimental Field Trials of Genetically Engineered Crops to Related Wild Plants.* Center for Food Safety, Washington DC, USA. http://www.centerforfoodsafety.org/pubs/Contaminating_the_Wild_Report.pdf

Gustafson DI, **Horak** MJ, **Rempel** CB, **Metz** SG, **Gigax** DR and **Hucl** P 2005. An empirical model for pollen-mediated gene flow in wheat. *Crop Science* 45: 1286–1294. http://crop.scijournals.org/cgi/reprint/45/4/1286

Hails RS and **Morley** K 2005. Genes invading new populations: A risk assessment perspective. *TRENDS in Ecology and Evolution* 20: 245–252. DOI: 10.1016/j.tree.2005.02.006

Hall L, **Topinka** K, **Huffman** J, **Davis** L and **Good** A 2000. Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant B. napus volunteers. *Weed Science* 48: 688–694. DOI: 10.1614/0043-1745(2000)048[0688:PFBHRB]2.0.CO;2

Hani AA, **Dwyer** J, **Moloney** M, **Gressel** J 2006. Mitigation of establishment of *Brassica napus* transgenes in volunteers using a tandem construct containing a selectively unfit gene. *Plant Biotechnology Journal* 4: 7–21. DOI: 10.1111/j.1467-7652.2005.00152.x

Hani AA, **Galili** S and **Gressel** J 2004. Tandem constructs to mitigate transgene persistence: Tobacco as a model. *Molecular Ecology* 13(3): 697–710. DOI:

10.1046/j.1365-294X.2004.02092.x

Hani AA, **Galili** S and **Gressel** J 2005. Poor competitive fitness of transgenically mitigated tobacco in competition with the wild type in a replacement series. *Planta* 222: 372–385. DOI: 10.1007/s00425-005-1540-6

Hansen G and **Wright** MS 1999. Recent advances in the transformation of plants. *TRENDS in Plant Science*, 4: 226-231. DOI: 10.1016/S1360-1385(99)01412-0

Hansona BD, **Mallory-Smithb** CA, **Shafiic** B, **Thillc** DC and **Zemetrac** RS 2005. Pollen-mediated gene flow from blue aleurone wheat to other wheat cultivars. *Crop Science* 45: 1610–1617. http://crop.scijournals.org/cgi/reprint/45/4/1610.pdf

Hartl DL and **Clark** AG 1989. *Principle of Population Genetics.* 2nd Edition. Sinauer Associates Inc., Sunderland MA, USA.

Hasler CM 2000. The changing face of functional foods. *Journal of the American College of Nutrition* 19: 499S–506S. http://www.jacn.org/cgi/reprint/19/suppl_5/499S

Hatti-Kaul R, **Tornvall** U, **Gustafsson** L and **Borjesson** P 2007. Industrial biotechnology for the production of bio-based chemicals: A cradle-to-grave perspective. *TRENDS in Biotechnology* 25 (3): 119–124. DOI: 10.1016/j.tibtech.2007.01.001

Haygood R, **Ives** RA and **Andow** DA 2003. Consequences of recurrent gene flow from crops to wild relatives. *Proceedings of the Royal Society: Biological Sciences* 270: 1879–1886. DOI: 10.1098/rspb.2003.2426

Heinemann JA 2007. *A Typology of the Effects of (Trans)Gene Flow on the Conservation and Sustainable Use of Genetic Resources.* Commission on Genetic Resources for Food and Agriculture, Background Study Paper Number 35 Rev. 1, Food and Agriculture Organization of the United Nations, Rome, Italy, June 2007. ftp://ftp.fao.org/ag/cgrfa/bsp/bsp35r1e.pdf

Hoagland RE and **Paul** RV 1978. A comparative SEM study of red rice and several commercial rice (*Oryza sativa*) varieties. *Weed Science* 26(6): 619–625

Hokanson SC, **Grumet** R and **Hancock** JF 1997. Effect of border rows and trap/donor ratios on pollen-mediated gene movement. *Ecology Applications* 7(3): 1075–1081. DOI: 10.1890/1051-0761(1997)007[1075:EOBRAT]2.0.CO;2

Hooftman DAP, **Jong** MJD, **Oostermeijer** JGB and den Nijs HJCM 2007. Modelling the long-term consequences of crop-wild relative hybridization: A case study using four generations of hybrids. *Journal of Applied Ecology* 44(5): 1035–1045. DOI: 10.1111/j.1365-2664.2007.01341.x

Horlick-Jones T, **Rowe** G and **Walls** J 2007. Citizen engagement processes as information systems: The role of knowledge and the concept of translation quality. *Public Understanding of Science* 16: 259–278. DOI: 10.1177/0963662506074792

Hothorn LA and **Oberdoerfer** R 2006. Statistical analysis used in the nutritional assessment of novel food using the proof of safety. *Regulatory Toxicology and Pharmacology* 44: 125–135. DOI: 10.1016/j.yrtph.2005.10.001

Huang CY, **Ayliffe** MA and **Timmis** JN 2003. Direct measurement of the transfer rate of chloroplast DNA into the nucleus. *Nature* 422, 72–76. DOI: 10.1038/nature01435

Huang J, Hu R, **Rozelle** R and **Pray** C 2005. Insect-resistance GM rice in farmers' fields: Assessing productivity and health effects in China. *Science* 308: 688–690. DOI: 10.1126/science.1108972

Huffman WE, **Rousu** M, **Shogren** JF and **Tegene** A 2007. The effects of prior beliefs and learning on consumers' acceptance of genetically modified foods. *Journal of Economic Behavior and Organization* 63: 193–206. DOI: 10.1016/j.jebo.2005.04.019

IRRI 2000. *Wild and Weedy Rice in Rice Ecosystems in Asia - a Review*. Limited Proceedings Series No. 2. Baki BB, Chin DV & Mortimer AM (eds), International Rice Research Institute (IRRI), The Philippines. http://www.irri.org/publications/limited/pdfs/weedyrice.pdf

James C 2006. Global Status of Commercialized Biotech/GM Crops: 2006. ISAAA Brief No. 35. International Service for the Acquisition of Agri-Biotech Applications (ISAAA), Ithaca NY, USA

Jarvis DI and **Hodgkin** T 1999. Wild relatives and crop cultivars: detecting natural introgression and farmer selection of new genetic combinations in agroecosystems. *Molecular Ecology* 8(s1): S159–173. DOI:10.1046/j.1365-294X.1999.00799.x

Jenczewski E, **Ronfort** J and **Chèvre** AM 2002. Crop-to-wild gene flow, introgression and possible fitness effects of transgenes. *Environment Biosafety Research* 2: 9–24. DOI: 10.1051/ebr:2003001

Johnson KL, **Raybould** AF, **Hudson** MD and **Poppy** GM 2007. How does scientific risk assessment of GM crops fit within the wider risk analysis? *TRENDS in Plant Science* 12: 1–5. DOI: 10.1016/j.tplants.2006.11.004

Johnston WJ, **Vu** DQ, **Giband** M, **Lu** BR, **Andow** DA, **Nguyen** HH, **Vuong** TP and **Le** QQ 2008. Environmental risks associated with gene flow from transgenic cotton in Viet Nam. In: *Environmental Risk Assessment of Genetically Modified Organisms: Challenges and Opportunities with Bt Cotton in Viet Nam*. Andow DA, Hilbeck A & Nguyen Van Tuat (eds), CABI Publishing, Wallingford, UK. pp.

Kaul MLH 1988. *Male Sterility in Higher Plants*. Springer-Verlag, Berlin, Germany. pp. 1005.

Khan MS and **Maliga** P 1999. Fluorescent antibiotic resistance marker for tracking plastid transformation in higher plants. *Nature Biotechnology* 17: 910–915. DOI: 10.1038/12907

Kiang YT, **Antonvics** J and **Wu** L 1979. The extinction of wild rice (*Oryza perennis formosa*) in Taiwan. *Journal of Asian Ecology* 1: 1–9.

Knight JG, **Mather** DW, **Holdsworth** DK and **Ermen** DF 2007. Acceptance of GM food - An experiment in six countries. *Nature Biotechnology* 25: 507–508. DOI: 10.1038/nbt0507-507

König A, **Cockburn** A, **Crevel** RWR, **Debruyne** E, **Grafstroem** R, **Hammerling** U, **Kimber** I, **Knudsen** I, **Kuiper** HA, **Peijnenburg** AACM, **Penninks** AH, **Poulsen** M, **Schauzu** M and **Wal** JM 2004. Assessment of the safety of foods derived from genetically modified (GM) crops. *Food and Chemical Toxicology*, 42: 1047–1088. DOI: 10.1016/j.fct.2004.02.019

Koopman WJM, **Li** YH, **Coart** E, **De Weg** EV, **Vosman** B, **Roldan-Ruiz** I and **Smulders** MJM 2007. Linked vs. unlinked markers: Multilocus microsatellite haplotype-sharing as a tool to estimate gene flow and introgression. *Molecular Ecology* 16: 243–256. DOI: 10.1111/j.1365-294X.2006.03137.x

Kooter JM, **Matzke** MA and **Meyer** P 1999. Listening to the silent genes: Transgene silencing, gene regulation and pathogen control. *TRENDS in Plant Science* 4: 340–347. DOI: 10.1016/S1360-1385(99)01467-3

Kowalchuk GA, **Bruinsma** M and **Van Veen** JA 2003. Assessing responses of soil microorganisms to GM plants. *TRENDS in Ecology and Evolution* 18: 403–410. DOI: 10.1016/S0169-5347(03)00187-3

Kwon SL, **Smith** RJ Jr and **Talbert** RE 1991. Interference of red rice (*Oryza sativa*) densities in rice (*O. sativa*). *Weed Science* 39, 169–174.

Lavigne C, **Klein** EK, and **Couvet** D 2002. Using seed purity data to estimate an average pollen mediated gene flow from crops to wild relatives. *Theoretical and Applied Genetics* 104: 139–145. DOI: 10.1007/s001220200017

Lee SM, **Kang** KS, **Chung** H, **Yoo** SH, **Xu** XM, **Lee** SB, **Cheong** JJ, **Daniell** H and **Kim** M 2006. Plastid transformation in the monocotyledonous cereal crop, rice (*Oryza sativa*) and transmission of transgenes to their progeny. *Molecules and Cells* 21: 401–410. http://www.molcells.org/home/journal/include/downloadPdf.asp?articleuid=%7B54FF4139%2D8199%2D4E7C%2D9FDE%2DCFD9D201D030%7D

Li GP, **Wu** KM, **Gould** F, **Wang** JK, **Miaoi** J, **Gao** XW and **Guo** YY 2007. Increasing tolerance to *Cry1Ac* cotton from cotton bollworm, *Helicoverpa armigera*, was confirmed in *Bt* cotton farming area of China. *Ecological Entomology* 32: 366–375. DOI: 10.1111/j.1365-2311.2007.00891.x

Linder CR and **Schmitt** J 1994. Assessing the risks of transgene escape through time and crop-wild hybrid persistence. *Molecular Ecology* 3: 23–30.

Linder CR, **Taha** I, **Seiler** GJ, **Snow** AA and **Rieseberg** LH 1998. Long-term introgression of crop genes into wild sunflower populations. *Theoretical and Applied Genetics* 96: 339–347. DOI: 10.1007/s001220050746

Losey JE, **Rayor** LS and **Carter** ME 1999. Transgenic pollen harms monarch larvae. *Nature* 399: 214. DOI: 10.1038/20338

Lu B-R 1993. Biosystematic Investigations of Asiatic Wheatgrass – *Elymus* L. (Triticeae: Poaceae). PhD thesis, Department of Plant Breeding Research, Swedish University of Agricultural Sciences, Svalöv, Sweden.

Lu B-R 2003. Transgene containment by molecular means-is it possible and cost effective? *Environmental Biosafety Research* 2: 3–8. http://www.ebr-journal.org/index.php?option=article&access=standard&Itemid=129&url=/articles/ebr/pdf/2003/01/01.pdf

Lu B-R 2004. Conserving biodiversity of soybean gene pool in the biotechnology era. *Plant Species Biology* 19: 115–125. DOI: 10.1111/j.1442-1984.2004.00108.x

Lu B-R, 2006. Identifying possible environmental hazard from GM rice in China to inform biosafety assessment. In: *Proceedings of 9th International Symposium on the Biosafety of Genetically Modified Organisms*, 24-29 Sept. 2006, Jeju Island, South Korea, pp. 106–110. http://www.isbr.info/isbgmo/docs/9th_isbgmo_program.pdf

Lu B-R and **Snow** AA 2005. Gene flow from genetically modified rice and its environmental consequences. *BioScience* 55: 669–678. DOI: 10.1641/0006-3568(2005)055[0669:GFFGMR]2.0.CO;2

Lu B-R, **Salomon** B and **von Bothmer** R 1991. Meiotic studies of the progenies from intergeneric crosses *Elymus × Hordeum and Elymus × Secale. Genome* 33: 425–432. DOI: 10.1139/g90-064

Luna VS, **Figueroa** JM, **Baltazar** BM, **Gomez** RL, **Townsend** R and **Schoper** JB 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Science* 41:1551–1557. http://crop.scijournals.org/cgi/reprint/41/5/1551.pdf

Lutz KA, **Knapp** JE and **Maliga** P 2001. Expression of bar in the plastid genome confers herbicide resistance. *Plant Physiology* 125: 1585–1590. http://www.plantphysiol.org/cgi/reprint/125/4/1585.pdf

Madsen KH, **Valverde** BE and **Jensen** JE 2002. Risk assessment of herbicide resistant crops: A Latin American perspective using rice (*Oryza sativa*) as a model. *Weed Technology* 16: 215–223.

Marshall A 2007. GM soybeans and health safety - a controversy reexamined. *Nature Biotechnology* 25: 981–987. DOI: 10.1038/nbt0907-981

Marvier M and **Van Acker** RC 2005. Can crop transgenes be kept on a leash? *Frontiers in Ecology and the Environment* 3: 99–106. http://www.scu.edu/cas/biology/staffandfaculty/upload/Marvier%20&%20VanAcker%20low%20res.pdf

Matsuoka T, **Kuribara** H, **Takubo** K, **Akiyama** H, **Miura** H, **Goda** Y, **Kusakabe** Y, **Isshiki** K, **Toyoda** M and **Hino** A 2002. Detection of recombinant DNA segments introduced to genetically modified maize (*Zea mays*). *Journal of Agriculture and Food Chemistry*, 50: 2100–2109. DOI: 10.1021/jf011157t

McBride KE, **Svad** Z, **Schaaf** DJ, **Hogan** PS, **Stalker** DM and **Maliga** P 1995. Amplification of a chimeric bacillus gene in chloroplasts leads to an extraordinary level of an insecticidal protein in tobacco. *Nature Biotechnology* 13: 362–365. DOI: 10.1038/nbt0495-362

Mercer KL, **Shaw** RG, **Wyse** DL 2006. Increased germination of diverse crop-wild hybrid sunflower seeds. *Ecological Applications* 16: 845–854. http://www.biosci.ohio-state.edu/~asnowlab/Mercer06sunflogerm.pdf

Mercer KL, **Andow** DA, **Wyse** DL and **Shaw** RG. 2007. Stress and domestication traits increase the relative fitness of crop-wild hybrids in sunflower. *Ecology Letters* 10: 383–393. http://www.biosci.ohio-state.edu/~asnowlab/Merceretal07.pdf

Messeguer J, **Fogher** C, **Guiderdoni** E, **Marfa** V, **Catala** MM, **Baldi** G and **Mele** E 2001. Field assessment of gene flow from transgenic to cultivated rices (*Oryza sativa* L.) using a herbicide resistance genes as tracer marker. *Theoretical and Applied Genetics* 103: 1151–1159. DOI: 10.1007/s001220100713

Mogensen H and **Rusche** M 2000. Occurrence of plasmids in rye sperm cells. *American Journal of Botany* 87: 1189–1192. http://www.amjbot.org/cgi/reprint/87/8/1189

Nap JP, **Metz** PLJ, **Escaler** M and **Conner** AJ 2003. The release of genetically modified crops into the environment - Part I. Overview of current status and regulations. *Plant Journal* 33: 1–18. DOI: 10.1046/j.0960-7412.2003.01602.x

Novoselova TA, **Meuwissen** MPM and **Huirne** RBM 2007. Adoption of GM technology in livestock production chains: an integrating framework. *TRENDS in Food Science and Technology* 18: 175–188. DOI: 10.1016/j.tifs.2006.12.005

O'Callaghan M, **Glare** TR, **Burgess** EPJ, **Malone** LA 2005. Effects of plants genetically modified for insect resistance on non-target organisms. *Annual Review of Entomology* 50: 271–292. DOI: 10.1146/annurev.ento.50.071803.130352

Oliveira AR, **Castro** TR, **Capalbo** DMF and **Delalibera** I 2007. Toxicological evaluation of genetically modified cotton (Bollgard®) and Dipel ® WP on the non-target soil mite *Scheloribates praeincisus* (Acari: Oribatida). *Experimental and Applied Acarology* 41: 191–201. DOI: 10.1007/s10493-007-9059-0

Olofsdotter M, **Valverde** BE and **Madsen** KH 2000. Herbicide resistant rice (*Oryza sativa L.*): Global implications for weedy rice and weed management. *Annals of Applied Biology* 137: 279–295. DOI: 10.1111/j.1744-7348.2000.tb00069.x

Ortiz-Garcia S, **Ezcurra** E, **Schoel** B, **Acevedo** F, **Soberon** J and **Snow** AA 2005. Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003-2004). *Proceedings of the National Academy of Sciences USA* 102: 12338–12343. DOI: 10.1073/pnas.0503356102

Oszvald M, **Kang** TJ, **Tomoskozi** S, **Tamas** C, **Tamas** L, **Kim** TG and **Yang** MS 2007. Expression of a synthetic neutralizing epitope of porcine epidemic diarrhea virus fused with synthetic B subunit of *Escherichia coli* heat labile enterotoxin in rice endosperm. *Molecular Biotechnology* 35: 215–233. DOI: 10.1016/j.vaccine.2005.01.027

Phipps RH, **Deaville** ER and **Maddison** BC 2003. Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. *Journal of Dairy Science* 86: 4070–4078. http://jds.fass.org/cgi/reprint/86/12/4070

Pilson D and **Prendeville** HR 2004. Ecological effects of transgenic crops and the escape of transgenes into wild populations. *Annual Review of Ecology Evolution and Systematics* 35: 149–174. DOI: 10.1146/annurev. ecolsys.34.011802.132406

Poppy G 2000. GM crops: Environmental risks and non-target effects. *TRENDS in Plant Science* 5: 4–6. DOI: 10.1016/S1360-1385(99)01514-9

Pray C, **Huang** JK, **Hu** RF and **Rozelle** S 2002. Five years of Bt cotton in China - the benefits continue. *The Plant Journal* 31: 423–430. DOI: 10.1046/j.1365-313X.2002.01401.x

Prescott VE, **Campbell** PM, **Moore** A, **Mattes** J, **Rothenberg** ME, **Foster** PS, **Higgins** TJV and **Hogan** SP 2005. Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. *Journal of Agriculture and Food Chemistry* 53: 9023–9030. DOI: 10.1021/jf050594v

Pretty J 2001. The rapid emergence of genetic modification in world agriculture: Contested risks and benefits. *Environmental Conservation* 28: 248–262. DOI: 10.1017/S0376892901000261

Quist D and **Chapela** I 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414: 541–543. DOI: 10.1038/35107068

Ramjoue C 2007. The transatlantic rift in genetically modified food policy. *Journal of Agriculture and Environmental Ethics* 20: 419–436. DOI: 10.1007/s10806-007-9047-8

Rao KV, **Rathore** KS, **Hodges** TK, **Fu** X, **Stoger** E, **Sudhakar** D, **Williams** S, **Christou** P, **Bharathi** M, **Bown** DP, **Powell** KS, **Spence** J, **Gatehouse** AMR and **Gatehouse** JA 1998. Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. *Plant Journal* 15: 469–477. DOI: 10.1046/j.1365-313X.1998.00226.x

Raven PH 2005. Transgenes in Mexican maize: Desirability or inevitability? *Proceedings of the National Academy of Sciences USA* 102: 13003–13004. http://www.pnas.org/cgi/reprint/0506082102v1.pdf

Repellin A, **Baga** M, **Jauhar** PP and **Chibbar** RN 2001. Genetic enrichment of cereal crops via alien gene transfer: New challenges. *Plant Cell, Tissue and Organ Culture* 64: 159–183. DOI: 10.1023/A:1010633510352

Rieger MA, **Lamond** M, **Preston** C, **Powles** SB and **Roush** RT 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296: 2386–2388. DOI: 10.1126/science.1071682

Rieger MA, **Potter** TD, **Preston** C and **Powles** SB 2001. Hybridization between *Brassica napus* L. and *Raphanus raphanistrum L.* under agronomic field conditions. *Theoretical and Applied Genetics* 103: 555–560. DOI: 10.1007/PL00002909

Rieseberg LH, **Church** SA and **Morjan** CL 2004. Integration of populations and differentiation of species. *New Phytologist* 161: 59–69. DOI: 10.1046/j.1469-8137.2003.00933.x

Rong J, **Lu** B-R, **Song** ZP, **Su** J, **Snow** AA, **Zhang** XS, **Sun** SG, **Chen** R and **Wang** F 2007. Dramatic reduction of crop-to-crop gene flow within a short distance from transgenic rice fields. *New Phytologist* 173: 346–353. DOI: 10.1111/j.1469-8137.2006.01906.x

Rong J, **Song** ZP, **Su** J, **Xia** H, **Lu** B-R and **Wang** F 2005. Low frequency of transgene flow from *Bt/CpTI* rice to its non-transgenic counterparts planted at close spacing. *New Phytologist* 168: 559–566. DOI: 10.1111/j.1469-8137.2005.01539.x

Rong J, **Xia** H, **Zhu** YY, **Wang** YY and **Lu** B-R 2004. Asymmetric gene flow between traditional and hybrid rice varieties (*Oryza sativa*) estimated by nuclear SSRs and its implication in germplasm conservation. *New Phytologist* 163: 439–445. DOI: 10.1111/j.1469-8137.2004.01100.x

Saji H, **Nakajima** N, **Aono** M, **Tamaoki** M, **Kubo** A, **Wakiyama** S, **Hatase** Y and **Nagatsu** M 2005. Monitoring the escape of transgenic oilseed rape around Japanese ports and roadsides. *Environmental Biosafety Research* 4: 217–222. DOI: 10.1051/ebr:2006003

Sanvido O, **Romeis** J and **Bigler** F 2007. Ecological impacts of genetically modified crops: Ten years of field research and commercial cultivation. In: *Green Gene Technology. Research in an Area of Social Conflict*, Fiechter

A & Sautter C (eds). Series: *Advances in Biochemical Engineering/ Biotechnology* 107: 235–278. DOI: 10.1007/10_2007_048

Schilter B and **Constable** A 2002. Regulatory control of genetically modified (GM) foods: Likely developments. *Toxicology Letters* 127: 341–349. DOI: 10.1016/S0378-4274(01)00518-5

Scott SE and **Wilkinson** MJ 1999. Low probability of chloroplast movement from oilseed rape (*Brassica napus*) into wild *Brassica rapa*. *Nature Biotechnology* 17: 390 – 392. DOI: 10.1038/7952

Sears ER 1983. The transfer to wheat of interstitial segments of alien chromosomes. In: *Proceedings of the 6th International Wheat Genetics Symposium*, 28 November – 3 December 1983, Kyoto, Japan. Sakamoto S (ed), Plant Germplasm Institute, Faculty of Agriculture, Kyoto University, Kyoto, Japan. pp. 5–12.

Shepherd DN, **Mangwende** T, **Martin** DP, **Bezuidenhout** M, **Kloppers** FJ, Carolissen CH, Monjane AL, Rybicki EP and Thomson JA 2007. Maize streak virus-resistant transgenic maize: A first for Africa. *Plant Biotechnology Journal* 5: 759–767. DOI: 10.1111/j.1467-7652.2007.00279.x

Sims SR and **Berberich** SA 1996. *Bacillus thuringiensis* CryIA protein levels in raw and processed seed of transgenic cotton: Determination using insect bioassay and ELISA. Journal of *Economic Entomology* 89: 247–251.

Snow AA, **Andow** DA, **Gepts** P, **Hallerman** EM, **Power** A, **Tiedje** JM and **Wolfenbarger** LL 2005. Genetically modified organisms and the environment: Current status and recommendations. *Ecology Applications* 15: 377–404. DOI: 10.1890/04-0539

Snow AA, **Pilson** D, **Rieseberg** LH, **Paulsen** MJ, **Pleskac** N, **Reagon** MR, **Wolf** DE and **Selbo** SM 2003. A *Bt* transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecology Applications* 13: 279–286. DOI: 10.1890/1051-0761(2003)013[0279:ABTRHA]2.0.CO;2

Song ZP, **Lu** B-R and **Chen** J K 2001. A study of pollen viability and longevity in *Oryza rufipogon, O. sativa* and their hybrid. *International Rice Research Notes* 26: 31–32. http://www.irri.org/publications/irrn/pdfs/vol26no2/

irrn262genetic.pdf

Song ZP, **Lu** B-R and **Chen** JK 2004a. Pollen flow of cultivated rice measured under experimental conditions. *Biodiversity and Conservation* 13: 579–590. DOI: 10.1023/B:BIOC.0000009491.24573.1d

Song ZP, **Lu** B-R, **Wang** B and **Chen** JK 2004b. Fitness estimation through performance comparison of F1 hybrids with their parental species *Oryza rufipogon* and *O. sativa. Annals of Botany* 93: 311–316. DOI:10.1093/aob/mch036

Song ZP, **Lu** B-R, **Zhu** YG. and **Chen** JK 2003. Gene flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. *New Phytologist* 157: 657–665. DOI: 10.1046/j.1469-8137.2003.00699.x

Spök A 2007. Molecular farming on the rise - GMO regulators still walking a tightrope. *Trends in Biotechnology* 25: 74–82. DOI: 10.1016/ j.tibtech.2006.12.003

Sripaoraya S, **Keawsompong** S, **Insupa** P, **Power** JB, **Davey** MR and **Srinives** P 2006. Genetically manipulated pineapple: Transgene stability, gene expression and herbicide tolerance under field conditions. *Plant Breeding* 125: 411–413. DOI: 10.1111/j.1439-0523.2006.01229.x

Stewart CN, All JN, **Raymer** PL and **Ramachandran** S 1997. Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. *Molecular Ecology* 6: 773–779. DOI: 10.1046/j.1365-294X.1997.00239.x

Stewart CN, **Halfhill** MD and **Warwick** SI 2003. Transgene introgression from genetically modified crops to their wild relatives. *Nature Reviews of Genetics* 4: 806–817. DOI: 10.1038/nrg1179 http://plantsciences.utk.edu/ pdf/stewartnrgintrogression.pdf

Stewart CN, **Richards** HA and **Halfhill** MD 2000. Transgenic plants and biosafety: Science, misconceptions and public perceptions. *Biotechniques* 29: 832.

Svab Z, **Hajdukiewicz** P and **Maliga** P 1990. Stable transformation of plastids in higher plants. *Proceedings of the National Academy of Sciences USA* 87: 8526–8530. http://www.pnas.org/cgi/reprint/87/21/8526

Tang W, **Newton** RJ, **Lin** JX and **Charles** TM 2006. Expression of a transcription factor from *Capsicum annuum* in pine calli counteracts the inhibitory effects of salt stress on adventitious shoot formation. *Molecular Genetics and Genomics* 276: 242–253. DOI: 10.1007/s00438-006-0137-5

Thomson JA 2001. Horizontal transfer of DNA from GM crops to bacteria and to mammalian cells. *Journal of Food Science* 66: 188–193. DOI: 10.1111/j.1365-2621.2001.tb11314.x

Toyama K, **Bae** CH, **Kang** JG, **Lim** YP, **Adachi** T, **Riu** KZ, **Song** PS and **Lee** HY. 2003. Production of herbicide-tolerant zoysiagrass by *Agrobacterium*-mediated transformation. *Molecules and Cells* 16: 19–27. http://www.molcells.org/home/journal/include/downloadPdf.asp?articleuid=%7B1B50E297-94FA-4431-B655-7916159C89AD%7D

Vanderschuren H, **Stupak** M, **Futterer** J, **Gruissem** W and **Zhang** P 2007. Engineering resistance to geminiviruses - review and perspectives. *Plant Biotechnology Journal* 5: 207–220. DOI: 10.1111/j.1467-7652.2006.00217.x

Vaughan DA 1994. *Wild Relatives of Rice: A Genetic Resources Handbook*. International Rice Research Institute (IRRI), The Philippines. pp. 137.

Vogel G. 2006. Genetically modified crops - Tracing the transatlantic spread of GM rice. Science 313: 1714–1714. DOI: 10.1126/science.313.5794.1714a

Wang F, **Yuan** QH, **Shi** L, **Qian** Q, **Liu** WG, **Kuang** BG, **Zeng** DL, **Liao** YL, **Cao** B and **Jia** SR 2006. A large-scale field study of transgene flow from cultivated rice (*Oryza sativa*) to common wild rice (*O. rufipogon*) and barnyard grass (*Echinochloa crusgalli*). *Plant Biotechnology Journal* 4: 667–676. DOI: 10.1111/j.1467-7652.2006.00210.x

Wang RRC, **Liu** ZW, **Carman** JG 1993. The introduction and expression of apomixes in hybrids of wheat and *Elymus rectisetus*. In: *Proceedings of the 8th International Wheat Genetics Symposium*, 20–25 July 1993, Beijing, PR China. Li ZS & Xin ZY (eds), Agricultural Scientech Press, Beijing, PR China. Vol. 1, pp. 317–319

Watrud LS, **Lee** EH, **Fairbrother** A, **Burdick** C, **Reichman** JR, **Bollman** M, **Storm** M, **King** G and **Van de Water** PK 2004. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with

CP4 EPSPS as a marker. *Proceedings of the National Academies of Science USA* 101: 14533–14538. http://www.pnas.org/cgi/reprint/101/40/14533.pdf

Wilkinson MJ, **Davenport** IJ, **Charters** YM, **Jones** AE, **Allainguillaume** J, Butler HT, **Mason** DC and **Raybould** AF 2000. A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. *Molecular Ecology* 9: 983–991. DOI: 10.1046/j.1365-294x.2000.00986.x

Williams IH 2002. The EU regulatory framework for GM crops in relation to bees. *Bee World* 83: 24–35.

Wu KM 2007. Monitoring and management strategy for *Helicoverpa armigera* resistance to *Bt* cotton in China. *Journal of Invertebrate Pathology* 95: 220–223. DOI: 10.1016/j.jip.2007.03.012

Yang PY, **Iles** M, **Yan** S and **Jolliffe** F 2005. Farmers' knowledge, perceptions and practices in transgenic *Bt* cotton in small producer systems in Northern China. *Crop Protection* 24: 229–239. DOI: 10.1016/j.cropro.2004.07.012

Ye X, **Al-Babili** S, **Klöti** A, **Zhang** J, **Lucca** P, **Beyer** P and **Potrykus** I 2000. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287: 303–305. DOI: 10.1126/science.287.5451.303

Zhao Y, **Qian** Q, **Wang** HZ and **Huang** DN 2007. Co-transformation of gene expression cassettes via particle bombardment to generate safe transgenic plant without any unwanted DNA. *In Vitro Cellular & Developmental Biology - Plant* 43: 328–334. DOI: 10.1007/s11627-007-9051-8