



## Research review paper

## Gene flow from genetically modified rice to its wild relatives: Assessing potential ecological consequences

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## ABSTRACT

Pollen-mediated gene flow is the major pathway for transgene escape from GM rice to its wild relatives. Transgene escape to wild *Oryza* species having AA-genome will occur if GM rice is released to environments with these wild *Oryza* species. Transgenes may persist to and spread in wild populations after gene flow, resulting unwanted ecological consequences. For assessing the potential consequences caused by transgene escape, it is important to understand the actual gene flow frequencies from GM rice to wild relatives, transgene expression and inheritance in the wild relatives, as well as fitness changes that brought to wild relatives by the transgenes. This article reviews studies on transgene escape from rice to its wild relatives via gene flow and its ecological consequences. A framework for assessing potential ecological consequences caused by transgene escape from GM rice to its wild relatives is discussed based on studies of gene flow and fitness changes.

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## 1. Introduction

The rapid development of biotechnology has greatly promoted the research and development of genetically modified (GM) crops worldwide. Consequently, a large number of transgenes conferring diverse traits have been successfully transferred into crop varieties through the transgenic biotechnology (Repellin et al., 2001; Lu and

Snow, 2005; Lee et al., 2006; Zhao et al., 2007). These traits include high protein contents and unique nutritional compounds (Gura, 1999; Hasler, 2000; Ye et al., 2000), disease and insect resistance (Datta et al., 1998; Huang et al., 2005; Bock, 2007), virus resistance (Shepherd et al., 2007), herbicide resistance (Lutz et al., 2001; Toyama et al., 2003), and salt and drought tolerances (Bahieldin et al., 2005; Tang et al., 2006). Likewise, in the process of research and development of GM rice, many beneficial traits with unique functions have been transferred into this crop by genetic engineering biotechnology (Table 1). The great success in transgenic biotechnology has

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**Table 1**  
Information of traits that have been transferred into cultivated rice (*O. sativa*) using genetic engineering biotechnology.

Trait	Gene	Donor	Transgenic product	Function	References
<i>Insect resistance</i>					
	<i>pinII</i>	Potato	Potato proteinase inhibitor II (pinII)	Inhibit trypsin and chymotrypsin simultaneously	Duan et al. (1996)
	<i>GNA</i>	Snowdrop	<i>Galanthus nivalis</i> agglutinin (GNA)	Have specificity to $\alpha$ -1,3- or 1,6-linked d-mannose residues in carbohydrates, is toxic to insects	Rao et al. (1998)
	<i>Cystatin</i>	Maize	Corn cystatin (CC)	Exhibit strong inhibitory activity against insect gut proteinases	Irie et al. (1996)
	<i>Cry1Ab</i>	<i>Bacillus thuringiensis</i>	Cry1Ab toxic protein	Bind reversibly to receptors on the surfaces of larval midgut and form ion channels	Shu et al. (2000)
	<i>Cry1Ac</i>	<i>B. thuringiensis</i>	Cry1Ac toxic protein	Binds reversibly to receptors on the surfaces of larval midgut and form ion channels	Nayak et al. (1997)
	<i>CpTI</i>	Cowpea	Cowpea trypsin inhibitor	Exhibit a strong inhibitory activity against trypsin	Xu et al. (1996)
<i>Disease resistance</i>					
	<i>Cecropin</i>	Silkworm ( <i>Bombyx mori</i> )	Cecropin B	Enhance resistance to bacterial leaf blight in rice	Sharma et al. (2000)
	<i>CHt-2</i> <i>CHt-3</i>	Rice	Chitinase	Enhance resistance to blast and sheath blight	Nishizawa et al. (1999)
	<i>Pib</i>	Rice		Enhance rice blast disease resistance	Wang et al. (1999)
	<i>Pi-ta</i>	Rice		Enhance rice blast disease resistance	Bryan et al. (2000)
	<i>Xa21</i>	<i>Oryza longistaminata</i>	Receptor kinase-like protein	Enhance resistance to bacterial blight	Song et al. (1995)
<i>Yield increase</i>					
	<i>Tzs, ipt</i>	<i>A. tumefaciens</i>	Isopentanyl transferase (IPT)	Stunted growth, loss of apical dominance, delayed senescence, and reduced root formation	Cao et al. (2004)
	<i>VHB</i>	<i>Vitreoscilla</i>	<i>Vitreoscilla hemoglobin</i> (VH)	VH facilitates oxygen transfer to the respiratory membranes	Cao et al. (2004)
<i>Quality improvement</i>					
Enhanced Vitamin A	<i>Psy, Crt1</i>	<i>Narcissu, pseudonarcissus, Erwinia uredovora</i>	Phytoene synthase and Phytoene dehydrogenase	Produce beta-carotene, a precursor of Vitamin A	Ye et al. (2000)
	Antisense <i>Wx</i> gene	Artificial synthetic		Introduce antisense <i>Waxy</i> gene to regulate <i>Waxy</i> gene expression, led to reduction in amylose and <i>Waxy</i> protein	Terada et al. (2000)
<i>Salt tolerance</i>					
	<i>BADH</i>	Sugar Beet	Betaine aldehyde dehydrogenase (BADH)	Synthesis of glycinebetaine into salt-sensitive crops leading to accumulation of glycinebetaine and improvement of salt tolerance	Guo et al. (1997)
	<i>mtID</i> <i>gutD</i>	<i>Escherichia coli</i>	Mannitol-1-phosphate dehydrogenase (mtID) Glucitol-6-phosphate dehydrogenase (gutD)	Produce and accumulate mannitol to increase the osmotic pressure, improve salt tolerance	Wang et al. (1999)
	<i>SsNHX1</i>	<i>Suaeda salsa</i>	<i>Suaeda salsa</i> vacuolar $\text{Na}^+/\text{H}^+$ antiporter	Removal of $\text{Na}^+$ from the cytoplasm by transporting it into vacuole via $\text{Na}^+/\text{H}^+$ exchangers	Zhao et al. (2006)
	<i>CMO</i>	Spinach	choline monoxygenase (CMO)	Accumulates glycinebetaine and improve salt tolerance	Shirasawa et al. (2006)
<i>Drought tolerant</i>					
	<i>SNAC1</i>	Rice		Delayed leaf-rolling and reduced rate of water loss	Hu et al. (2006)
	<i>MnSOD</i>	Pea	Manganese superoxide dismutase (MnSOD)	MnSOD evolved mechanisms to scavenge reactive oxygen species	Wang et al., 2005
	<i>codA</i>	<i>Arthrobacter globiformis</i>	Choline oxidase	catylases conversion of choline to glycine betaine that provides tolerance to drought stresses	Sakamoto et al. (1998)
<i>Herbicide resistance</i>					
	<i>Bar</i>	<i>Streptomyces hygroscopicus</i>	phosphinothricin-Acetyl transferase	Converts phosphinothricin (PPT) to a non-phytotoxic metabolite	Cao et al. (1992)
	<i>EPSPS</i>	<i>Agrobacterium sp.</i> strain CP4	5-enolpyruvylshikimate-3-phosphate synthase	Enhance Glyphosate resistance	Cao et al. (2004)

had a tremendous impact on the world crop production and cultivation patterns of agricultural species such as cotton, soybean, canola, and maize (James, 2007).

The commercial production of GM crops with various agronomically beneficial traits provides great opportunities for world's food security by enhanced efficiency of crop production. However, the extensive

environmental release and cultivation of GM crop varieties have also aroused enormous biosafety concerns and debates worldwide (Stewart et al., 2000; Ellstrand, 2001, 2003), including food and health safety (Cromwell et al., 2005; Hothorn and Oberdoerfer, 2006; Marshall, 2007), environmental safety (Conner et al., 2003; Pilon and Prendeville, 2004; Sanvido et al., 2007), as well as socio-economical and ethic

concerns (Finucane and Holup, 2005; Aerni, 2007; Einsele, 2007). Biosafety issue already becomes a crucial factor that has considerably constrained to the further development of transgenic biotechnology and the wider application of GM products in agriculture.

The most debated environmental biosafety issues include: 1) direct and indirect effects of toxic transgenes (e.g. the *Bt* insect-resistance gene) to non-target organisms (O'Callaghan et al., 2005; Oliveira et al., 2007); 2) influences of transgenes and GM plants on biodiversity, ecosystem functions, and soil microbes (Giovannetti et al., 2005; Oliveira et al., 2007); 3) transgene escape to crop landraces and wild relatives through gene flow and its potential ecological consequences (Wilkinson et al., 2000; Snow et al., 2003; Lu and Snow, 2005; Mercer et al., 2007); and 4) potential risks associated with the development of resistance to biotic-resistance transgenes in the target organisms (Dalecky et al., 2007; Li et al., 2007; Wu, 2007). In addition, there are still some unknown involvements in potentially significant interactions between transgenic traits and the environments.

Among the above environmental biosafety issues, transgene escape from a GM crop variety to its non-GM crop counterparts or wild relatives has aroused tremendous debates worldwide (Ellstrand et al., 1999; Ellstrand, 2001, 2003; Lu and Snow, 2005). This is because transgene escape can easily happen *via* gene flow that may result in potential ecological consequences if significant amount of transgenes constantly move to non-GM crops and wild relative species. This is particularly true when these transgenes can bring evolutionary selective advantages or disadvantages to crop varieties or wild populations. It is therefore essential to properly address the most relevant questions relating to the transgene outflow and its potential environmental consequences on a science based altitude.

Does transgene escape to wild relatives of a crop species *via* gene flow happen and at what frequencies? What are the potential biosafety impacts on environment caused by such gene flow? How can the assessment of potential environmental risks from transgene outflow be conducted under a biosafety framework? These questions should be addressed, not only for the benefit of scientists and researchers but importantly also for the decision-makers and consumers of the biotechnology products. The objective of this article is to introduce the concept of gene flow and the anticipated ecological consequences resulted from pollen-mediated transgene flow to wild relatives using rice as a model.

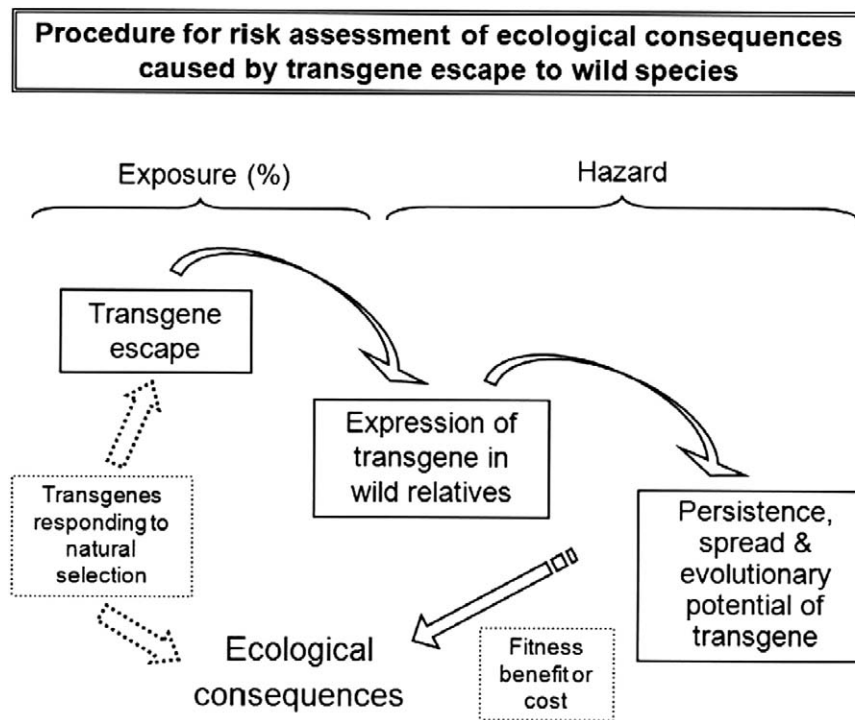
## 2. Transgene escape *via* pollen-mediated gene flow and its ecological consequences

Transgene escape means: a genetically modified gene moves from a GM crop to its non-GM crop counterparts (crop-to-crop) or wild relative species (crop-to-wild) *via* gene flow (Lu, 2008). Usually, there are three pathways for gene flow to occur: pollen-mediated (through outcrossing), seed-mediated (*via* seed dispersal), and vegetative propagule-mediated gene flow. Pollen-mediated gene flow commonly happens between sexually compatible individuals either within the same populations or among populations separated at certain distances. Wind, animal, water current and other factors can serve as media to promote pollen-mediated gene flow. Transgenes can escape from a GM crop to its wild relative species *via* pollen-mediated gene flow (Ellstrand et al., 1999; Lu and Snow, 2005). Frequencies of the pollen-mediated gene flow is primarily determined by the pollination biology of plant species, such as mating systems, amount of pollen produced by pollen donors and outcrossing rates of a pollen recipient. In addition, types of vectors for pollination and environmental conditions, such as the strength, and direction of wind, temperature, light intensity, and air humidity, will also influence pollen-mediated gene flow to a great extent. For example, as a wind-pollinated species, the frequency of pollen-mediated gene flow in rice is primarily determined by strength of wind, air humidity and temperature.

Pollen-mediated gene flow is a natural procedure in plant evolution, but in the case of GM crops, the movement of transgene(s) may have unintended ecological impacts when transgenic technology is extensively applied to crop production (Lu, 2008). Studies have shown that many crops can interbreed with their wild relatives (Ellstrand, 2003). Therefore, transgene escape *via* pollen-mediated gene flow from a GM crop to populations of wild or weedy relatives and its ecological impacts have been considerably discussed and debated. This is because wild or weedy plants that acquire transgenes will continue to evolve, subject to natural and artificial selection in the agricultural ecosystem and beyond, posing potential ecological consequences. Once transgenes have moved into populations of wild or weedy species, it is nearly impossible to remove them from the environments if the transgenes can persist and spread in the populations (West et al., 2008).

In principle, the magnitude of ecological consequences caused by transgene escape to wild relatives can be determined by the amount of transgenes that have flowed into the wild or weedy populations and the GM traits that have or do not have evolutionary advantages under natural selection. If the transgene conveys selectively advantageous or disadvantage traits, the flow of such a transgene into wild relatives may change the fitness of wild or weedy relatives. There are different possibilities for the fate of wild populations by incorporating a transgene. If the transgene can enhance the fitness of wild relatives with favorable traits such as pest resistance, drought tolerance, and enhanced growth ability, the transgene followed by gene flow would persist to and quickly spread in the populations of wild relatives through introgression. The individuals that have picked up the transgene will out compete with other individuals without the transgene under natural selection (Lu and Snow, 2005; Lu, 2008). This process will promote the fast increase of transgenic individuals and enhance their invasiveness, causing different degrees of weed problems by having the wild populations quickly expand to new territories. On the contrary, if the transgene reduces the fitness of wild relatives, the frequencies of individuals that contain the disadvantageous transgene will decrease gradually. This process will be accelerated by the recurrent gene flow and introgression from a neighboring GM crop, leading to the extinction of local populations by the so-called swarm effect (Ellstrand and Elam, 1993). In many parts of the world, populations of the wild relatives are surrounded by crop fields in agricultural ecosystems and bordering areas between agricultural lands and natural habitats (Wilkinson et al., 2000; Ellstrand et al., 2007), such swarm effect has already happened through crop-to-wild gene flow, even without the inclusion of transgenes (Kiang et al., 1979).

The movement of transgenes that convey traits resistant to biotic and abiotic stresses from GM rice to population of wild or weedy rice may accentuate the characteristics of weediness, which leads to stronger persistence and invasiveness of wild or weedy rice that are already existing weeds in rice ecosystems in many of countries (Delouche et al., 2007). On the other hand, GM rice may acquire genes for weediness from pollen-mediated gene flow of weedy or wild rice occurring inside or near GM rice fields, which leads to persistence and invasiveness of the GM rice, although the chance is low. Traits that may influence invasiveness include fertility, vegetative vigor, tolerance of a wide range of environmental conditions, and the quality and dispersal range of viable material. Therefore, the concerns of gene flow with respect to weediness are mostly related the following two aspects: 1) wild and weedy rice that invaded and persisted to the rice fields have the ability to become a more effective and aggressive weed; and 2) the GM rice volunteers or hybrids between GM rice and its wild relatives have the ability to become a more effective and aggressive weed, after incorporating transgenes that convey traits against biotic (insects or diseases) and abiotic (drought or herbicide) stresses. These concerns are under the hypothesis that a transgene from GM rice will bring fitness advantage to the populations of rice volunteer, weedy rice, and wild species (see, Ellstrand et al., 1999;



**Fig. 1.** Schematic illustration of three key steps for the assessment of potential ecological consequences caused by transgene escape from a GM crop to its wild relatives *via* pollen-mediated gene flow. The boxes with empty lines emphasize that only those transgenes with evolutionary selection advantages or disadvantages may pose potential ecological consequences by change of fitness in wild populations under different environment conditions. Therefore, the escape of such transgenes requires a thorough assessment (modified from Lu, 2008).

Ellstrand, 2001; Lu and Snow 2005). In addition, recurrent or sequential transgene flow from GM rice may cause transgene stacking in the same population of volunteer, weedy or wild rice. Also, weedy and wild rice have seeds with strong dormancy that can allow seed to persist in soil seed-bank for many years. All these make the prediction of the fate for escaped transgenes into the environment more complicated.

Another concern over transgene escape from GM rice to its wild relatives is for the consequences of genetic diversity. The presence of transgenes in the germplasm of wild rice relatives may represent 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, 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 like rice than in outcrossing species, which have greater opportunities for the mixing and dilution of crop alleles during sexual reproduction. 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).

### 3. Risk assessment of transgene escape and its ecological consequences

Gene flow is a natural process that happens all the time. Even for gene flow between the major domesticated crop species and their wild relatives, such a process has been continued for at least a few thousands of years, right from the onset of the domestication of wild ancestral species into cultivated forms. Therefore, gene flow *per se* is a widespread and natural event that should not be considered as potential ecological risk. To date, there has not been a large report on the major negative environmental consequences or disasters caused merely by gene flow.

However, if a gene confers environmentally selective advantages like the case in transgenes that have much stronger expression and a unique function brought by genetic modification (e.g. insect-resistant *Bt* or herbicide tolerant transgene), it might spread quickly through introgression of crop-weedy or crop-wild hybrids in the weedy and wild populations. Even with very low frequencies of gene flow from GM crops, a transgene can be rapidly accumulated and disseminated in weedy and wild populations under favorable selection (e.g., Ellstrand, 2003; Lu and Snow, 2005). The estimation of potential consequences caused by transgene escape into populations of wild and weedy species should take the selective advantage of specific transgenic traits into consideration, because different types of transgenic traits will have different effects to wild or weedy populations. Hypothetically, only the transgenes that have selective advantage in evolutionary process and can change the fitness of the recipient individuals or populations can persist or quickly spread in the wild or weedy recipient populations after the transgenes flow into the populations, leading to potential consequences. Therefore, the case-by-case principle should be applied rigidly for biosafety assessments of transgene escape and its environmental consequences, depending on the types of recipients (e.g., crops or wild species) that incorporate transgenes through gene flow.

#### 3.1. The risk assessment

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 assessment 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, to determine the quantitative data that associated with the risks is essential during the risk assessment exercises.

In the context of the environmental biosafety, the principle of risk assessment follows the equation: risk = hazard × exposure. Risk indicates the probability that any adverse effect occurs from an environmental hazard, and can be expressed as a percentage. 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 (e.g. environment or ecosystems).

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, to establish such knowledge is the 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 characterization (Andow and Zwahlen, 2006). Therefore, to design a protocol for environmental related biosafety risk assessment, one should consider the key factors and steps that can cause adverse or harmful effects by GM crop released into environments and the potential in which the adverse effects will occur.

### 3.2. A framework of risk assessment for transgene escape to wild relatives

To effectively assess environmental biosafety consequences created by transgene escape from GM rice to its wild relatives through pollen-mediated gene flow, it is necessary to attain knowledge that is relevant to the particular biosafety assessment, and to determine the knowledge gaps that are necessary to address the relevant scientific questions, following the principle of risk assessment. What is the possibility and frequency of a transgene flow from GM rice to its wild relatives? What is the fate of a transgene introgressed into populations of wild relatives? Can transgenes change the fitness of individuals of wild relatives? Can transgenes alter the demographical dynamics of a wild or weedy population? Does a transgene significantly enhance the invasiveness of wild individuals and populations? Correct answers to these questions will certainly help to close the knowledge gaps and facilitate the assessment of ecological risks caused by gene flow. Risk assessment is a procedure that helps to determine the occurrence and magnitude of relevant risks. For meeting such an objective of risk assessment, it is necessary to establish a general framework for determining whether environmental risks associated with transgene flow will occur, and how serious the risks will be at the various steps. There are three major steps closely associated with the rational assessment of transgene escape and its environmental consequences (Fig. 1).

First, understand transgene flow from GM rice to its wild relatives. If no such possibility for gene flow to happen 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, understand the transgene expression and inheritance in the hybrids between GM rice and wild relatives after gene flow. If a transgene can be incorporated into a wild individual through gene flow, but cannot express normally, there should be no, or very limited, ecological consequences after the transgene flow. Accordingly, no further risk assessment is required. Third, understand the fitness change caused by the introgression of transgene into wild individuals, and influence of dynamics of a wild population, which may bring about the increase in invasiveness of wild individuals or populations. Transgene escape and its potential ecological consequences will essentially depend on the success of the movement and spread of transgenes in a wild population through the successive procedures (Fig. 1). Based on the scheme of this framework, it is very important to collect sufficient base-line data and scientific research results relevant for assessing transgene escape and its ecological consequences in rice.

## 4. Cultivated rice and its wild or weedy relatives

Asian cultivated rice (*Oryza sativa*) was domesticated in the lower Yangtze River region (Zong et al., 2007). Another cultivated species, African cultivated rice (*O. glaberrima*), has its origin in West Africa and remains locally important in the areas (Chang, 1976). In addition to the two crops, there are more than 20 wild relatives in the genus *Oryza*, and about 50 other wild species in the tribe Oryzae of the grass family (Poaceae) in the rice gene pool (Vaughan, 1994). Species in the genus *Oryza* include different genome types, i.e., the AA, BB, CC, BBCC, CCDD, EE, FF, GG, and JJHH (Ge et al., 1999). *Oryza* species with different genome types have significant reproductive isolation (mostly genetic barriers), making them unlikely to hybridize with each other. Results from many experiments of artificial hybridization between *Oryza* species with different genomes have supported this statement (see Lu and Snow, 2005). Hybridization between species in different genera in the tribe Oryzae is also extremely difficult because of the strong genetic barriers. However, given the relative easiness of hybridization between the AA-genome *Oryza* species (Lu et al., 2000), gene flow from cultivated rice to its close relatives with the AA-genome is relatively easy, particularly from the crop to its direct wild ancestors and conspecific weedy types (Table 2). Therefore, gene flow between cultivated rice and the AA-genome species should be assessed most carefully, because many of these species grow in close proximity to cultivated rice within and around rice fields (Vaughan, 1994).

The six wild rice species containing the AA-genome can co-occur with the cultivated rice in different continents. All of the wild rice species have the potential to hybridize with the crop and with each other. Species with the AA-genome have relatively high sexual compatibility, complete chromosome pairing in meiosis of their F1 interspecific hybrids, and relatively high fertility of the F1 hybrids (Naredo et al., 1997, 1998; Lu et al., 1997, 1998, 2000). Weedy rice (*O. sativa* f. *spontanea*, also known as red rice) can easily hybridize with cultivated rice because it is the same biological species as the crop (Delouche et al., 2007). Weedy rice occurs in most major rice-producing regions of the world and is genetically variable. Wild and weedy rice can be very difficult to eradicate because many of their seeds disperse before the crop is harvested and accumulate in the soil seed-bank, while others can be collected by farmers and inadvertently planted with the next generation of crop seeds. All these evidently indicate the potential for transgenes in GM rice to escape to wild or weedy relatives through cross-pollination and persist through the survival of intra- and interspecific hybrids.

**Table 2**

Information of *Oryza* species containing the AA-genome (based on Lu, 1998; Lu and Silitonga, 1999; Vaughan, 1994).

Taxon	Habit	Life cycle	Distribution	Mating system
<i>O. sativa</i> L.	Cultivated	Biannual	Worldwide	Selfing
<i>O. glaberrima</i> Steud.	Cultivated	Annual	West Africa	Selfing
<i>O. rufipogon</i> Griff.	Wild	Perennial	Asia	Partially outbreeding
<i>O. nivara</i> Sharma et Shastry	Wild	Annual	Asia	Selfing
<i>O. longistaminata</i> A. Chev. et Roehr	Wild	Perennial	Africa	Self-incompatible
<i>O. barthii</i> A. Chev.	Wild	Annual	Africa	Selfing
<i>Oryza glumaepatula</i> Steud.	Wild	Perennial	Latin America	Selfing and partially outbreeding
<i>O. meridionalis</i> Ng	Wild	Annual	Northern Australia and New Guinea	Selfing
<i>O. sativa</i> f. <i>spontanea</i>	Weedy	Annual	Worldwide	Selfing

## 5. Gene flow in rice

In general, cultivated rice is characterized by high rates of self-pollination and very little cross-pollination between adjacent plants or fields (typically <1.0%). Experiments have shown that the frequency of pollen-mediated gene flow from GM rice lines to the adjacent non-GM counterpart was between 0.04% and 0.80% (Messeguer et al., 2001; Rong et al., 2004, 2005). This frequency can dramatically decrease by a spatial isolation between GM and non-GM rice lines for about a few meters (Rong et al., 2007). The low frequency of crop-to-crop gene flow in rice can only be used as a reference for predicting the amount of gene flow from rice to its wild relatives because our unpublished data from recent modeling studies indicated that the pollen load of a donor and outcrossing rates of a pollen recipient (wild or weedy rice) play an important role in determining the frequency of gene flow.

### 5.1. Gene flow from cultivated rice to wild rice

Several studies have detected evident gene flow from cultivated rice to wild rice (e.g., Song et al., 2003, Chen et al., 2004). Most of the work involves Asian species such as the perennial *Oryza rufipogon*, annual *Oryza nivara*, and intermediate forms of these taxa (e.g., Oka, 1988, Vaughan, 1994, Majumder et al., 1997). No data on experimental studies of gene flow between cultivated rice and native wild rice species in Africa, South America, or Australia have been published, but natural hybrid swarms have been reported there (Oka, 1988; Juliano et al., 1998; Naredo et al., 1998). Here, we only focus on gene flow from cultivated rice to the Asian *O. rufipogon* as a case study, because of the intensive studies of this species as an important genetic resource.

Compared with cultivated rice, *O. rufipogon* populations have a protracted flowering period, while the crop is much more synchronized within a given field. In southeast China, *O. rufipogon* usually flowers between October and January (B. R. Lu, pers. observation). Likewise, in southern Vietnam, this species typically flowers from November to February, although some plants flower as late as April or May (Cohen et al., 2008). With modern cultivars, flowering is independent of day-length and farmers can grow 2–3 cycles of rice crops per year in tropical and subtropical climates. Therefore, a subset of the annual cycle of rice crops is likely to overlap with the primary flowering period of *O. rufipogon*.

Rice pollen is remarkably short-lived (<30 minutes) (e.g., Song et al., 2001) and the maximum distance over which pollen flow was detected was 110 m (Song et al., 2004a). The daily patterns of flowering can also limit the extent of crop-wild gene flow. Daily flowering times can be shorter for cultivated rice than for wild rice, but they often overlap during the morning and late afternoon (Lu et al., 2003). Song et al. (2003) reported based on designed field experiments that the maximum frequency of gene flow to adjacent plants of *O. rufipogon* was up to 3%, and around 95% of the crop-wild hybrid seeds occurred on wild plants growing within 30 m of the crop. On the other hand, a gene flow rate of up to 18% from cultivated rice to a different *O. rufipogon* population at a close distance in China has been reported (Wang et al., 2006), suggesting that further studies of variation among populations are needed. Taken together, these studies indicate that pollen-mediated gene flow to *O. rufipogon* is quite limited in experiments that are designed to maximize opportunities for cross-pollination.

The persistence of crop genes into wild rice populations is influenced by the survival and fecundity of crop-wild hybrids, and by fitness effects of specific genes from the crop. In the case of *O. rufipogon*, F<sub>1</sub> hybrids have lower pollen fertility and lower seed set than wild genotypes although with great variation under different environmental conditions (e.g., Song et al., 2004a). This partial reproductive barrier may impede the rate at which crop genes introgress into wild populations, but it will not prevent introgression

altogether. Later generations of introgressed plants are expected to regain high fertility after deleterious crop genes have been lost or diluted. Song et al. (2004b) reported greater vegetative growth as compared to wild genotypes of *O. rufipogon*. This could be important in terms of introgression because *O. rufipogon* is a long-lived, clonally reproducing plant, and vigorous clones have the potential to competitively displace other clones in the same population.

### 5.2. Gene flow from cultivated rice to weedy rice

It is widely recognized that transgenes introduced into rice cultivars will escape to weedy rice populations, despite the fact that the rate of cross-pollination is quite low (e.g., Langevin et al., 1990). Cultivated rice and weedy rice are primarily self-pollinated and have short-lived pollen, but low levels of crop-to-weedy gene flow have been detected. For example, gene flow from experimental plots of herbicide-resistant GM rice to several weedy rice accessions ranged from 0.00 to 0.5% in Asia (Chen et al., 2004). Likewise, Shivrainia et al. (2007) reported comparable amount (0.003–0.008%) of crop-to-weedy gene flow at a much more extensive scale of field experiments in North America. Gealy et al. (2003) reviewed more than ten published studies of rates of gene flow from cultivated rice to weedy rice and noted that typical rates of crop-weed hybridization were in the range of 0.0–1.00%. Crop-weedy gene flow can occur in either direction Gealy et al. (2003), i.e., from crop-to-weed or weed-to-crop, and either direction of the gene flow will result in weedy hybrids possessing transgenes if GM rice is involved in gene flow. Therefore, rice genes (including transgenes) will introgress into weedy rice population through gene flow at rates that reflect the both frequency of hybridization and the net selective effects of these genes (e.g., Ellstrand, 2003).

The introgression and long-term persistence of crop genes in weedy rice populations is another important component of gene flow although limited studies have been conducted. One motivation for developing herbicide-resistant GM rice is to have a new option for managing rice field and saving labors. The intention is to adopt direct seeding and the abandon hand- or machine-transplanting of rice seedlings into flooded fields (Baki et al., 2000). This change may encourage introgression and long-term persistence of herbicide-resistance transgene moved into weedy rice populations, and will enhance infestations of weedy rice. Another factor that can affect introgression is enhanced fecundity of crop-weed hybrids due to the hybrid vigor (heterosis). Just as hybrid vigor is seen when inbred, cultivated lines are crossed to produce “hybrid weedy rice,” so may weedy rice benefit from hybridizing with the crop. When heterosis occurs, it could boost the frequency of crop alleles in weedy populations, including frequencies of transgenes.

## 6. Expression and inheritance of transgene in wild relatives of rice

A number of studies have demonstrated gene flow from cultivated rice to its wild or weedy populations, with considerable frequencies in many cases (Song et al., 2003; Chen et al., 2004; Wang et al., 2006). This indicates that transgene escape from GM rice to its wild relatives is unavoidable. To understand the expression and inheritance of escaped transgene is important for estimating ecological consequences that depends essentially on the ability in which the normal function of the transgene will be maintained in wild relative species. If the incorporated transgene cannot express normally in wild relative species, the transgene will not be able to alter the traits or fitness of the wild relatives. As a result, the transgene escape may not pose any ecological consequences. On the contrary, if the transgene can express normally, or even stronger than in the parental GM rice, and inherit normally in wild rice, the introgression of such transgene into wild rice may bring fitness change to the wild populations, causing unwanted ecological consequences, particularly from transgenes

that have obvious selective advantages for biotic (such as insect and disease) and abiotic (such as drought and salinity) stresses.

For the assessment of transgene expression, there are a number of methodologies to measure the content of transgenic protein, which includes ELISA (Sims and Berberich, 1996; Bashim et al., 2005), RT-PCR (Sripaoraya et al., 2006). Commonly, the principle of estimating transgene expression is to measure the amount 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. To estimate the expression of a transgene in common wild rice (*O. rufipogon*), we conducted an experiment to analyze the content of cryIaC protein in three GM rice lines that contained a *Bt* transgene, their F<sub>1</sub> hybrids with common wild rice and F<sub>2</sub> progeny at different growth stages (B. R. Lu, unpublished data). Based on the sandwich enzyme-linked immunosorbent assay (ELISA), we found that the average content of cryIaC protein in leaf samples of the wild rice populations ranged between 0.016% and 0.069% during the entire growth period, whereas that in stems varied between 0.12% and 0.39%. This study further indicated a great variation in cryIaC protein content among individuals of F<sub>1</sub> hybrids and F<sub>2</sub> progeny (0.0075%–0.22% in leaf and 0.054%–0.96% in stem samples), with some wild individuals showing dramatically higher level of *Bt* toxin than the cultivated GM rice. Results from this study suggested that the *Bt* transgene could express normally in individuals of wild rice and might have similar effects to the target insects as in GM rice. Based on the findings, we suggested that further assessments of environmental consequences caused by *Bt* transgene escape to wild rice should be conducted.

Similarly, the inheritance of a transgene in wild rice populations is important for predicting the fate of transgenes in the wild populations, and it can also be estimated by the production of artificial populations of F<sub>1</sub> hybrids and advanced progeny. The artificial populations can be achieved through crosses between GM rice and its wild relatives, and followed by consecutive backcrosses and self-pollination. If the transgene can be normally expressed in crop-wild hybrids and selfing/backcrossing progenies, as well as inherited between different generations, further biosafety assessment for ecological consequences should be conducted because the transgene can exert its usual function and passed on to populations of wild rice relatives.

## 7. Fitness of crop-wild and crop-weedy hybrid progeny

For the estimation of long-term persistence and spread of transgenes in wild rice population in relation to the fitness change, three key factors should be taken into consideration, given that knowledge of the close genetic relationships and high compatibility among the AA-genome *Oryza* species are obtained from previous studies: 1) the frequency of gene flow that allows transgenes being transferred to wild populations; 2) the fitness of early hybrids relative to their wild parents, and 3) possible fitness costs or benefits that are associated with a particular transgene (Jenczewski et al., 2002).

If a transgene can move from GM rice to its wild or weedy populations, and at the same time the incorporated transgene can be normally expressed and inherited in the wild relatives, it is then very important to continue risk assessment to understand whether or not the transgene will change the ecological and evolutionary fitness of individuals or populations of the wild relatives that have picked up the transgene. If transgene can change fitness of the wild relatives, the pattern of persistence and spread of the transgene in a wild or weedy rice population may vary significantly from the situation where no transgene is involved in introgression (Song et al., 2004b). Transgene may considerably alter the ability of wild or weedy rice in terms of its 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<sub>1</sub> hybrids, self-pollinated F<sub>2</sub>, F<sub>3</sub>

progenies, and backcrossed BC<sub>1</sub>, BC<sub>2</sub> progenies) under experimental conditions through artificial crosses between a GM crop and wild relatives will facilitate the data generation from fitness analysis for the biosafety assessment.

The common way to estimate fitness change is to examine the vegetative and reproductive productivity of crop-wild hybrids and progeny (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; Hani et al., 2005; Snow et al., 2003; Mercer et al., 2007). In a recent study Song et al. (2004b) estimated relative fitness of F<sub>1</sub> hybrids with their parental common wild rice (*O. rufipogon*) and cultivated rice through comparison of their field performance. In this study, field performance of the interspecific F<sub>1</sub> hybrids was estimated in comparison with their parental species, a cultivated rice (Minghui-63) and common wild rice under the controlled conditions. Results indicated that the F<sub>1</sub> hybrids had the lowest values of seedling survival ability, pollen viability, and seed production; intermediate values of seed germination, spikelet production and flag leaf areas; and the highest values of plant height, number of tillers and panicles, compared with their parents. Although the F<sub>1</sub> hybrids performed rather poorly at the stage of sexual reproduction, there were no significant differences in composite fitness across the whole life-history between the F<sub>1</sub> hybrids and their wild parental species. This study indicated that rice genes, including transgenes, may introgress into and persist to wild rice populations through vegetative and sexual reproduction. Further studies are required to examine the fitness of crop-wild hybrids including transgenes.

Transgene escape from GM rice to weedy rice populations through gene flow and further introgression of the transgene has also aroused great biosafety concerns (Chen et al., 2004; Cao et al., 2006), particularly for the herbicide-resistance transgene. Comparative fitness studies of crop-weedy hybrids containing transgenes with their weedy rice parents will facilitate the effective assessment of ecological consequences. Vegetative and reproductive traits relating to fitness were characterized in F<sub>1</sub> hybrids of three weedy rice strains crossed with two GM rice lines containing insect-resistance transgenes (*CpTI* or *Bt/CpTI*), under cultivation of pure or mixed design including the F<sub>1</sub> hybrids or/and weedy parents (B. R. Lu, unpublished data). Under a very minor insect pressure, the crop-weedy hybrids showed a better performance at the vegetative and reproductive stages, with taller plants, more tillers, panicles, and spikelets per plant, and higher 1000-seed weight than the weedy rice parents. However, the F<sub>1</sub> hybrid plants produced much less seeds than their weedy parents. The crop-weedy hybrids showed a generally enhanced relative fitness than the weedy rice parents, but this enhancement might be closely associated with the hybrid vigor rather than the fitness benefit brought by the transgenes. Nevertheless, we recognized that better seed quality as reflected by the 1000-grain weight and productive potential as reflected by the superior number of spikelets in F<sub>1</sub> hybrids may enhance the possibility of transgene introgression into weedy rice populations. The following-up analysis of F<sub>2</sub> populations with or without the transgenes did not show significant fitness benefit or cost as indicated by the same morphological and agronomical characters under similar field experimental designs and conditions, although the F<sub>2</sub> populations with the transgene consistently showed slightly better performance for some characters.

It is necessary however to point out that there are many biotic and abiotic parameters affecting the accurate estimation of fitness change in populations of wild and weedy rice caused by transgenes. This makes it very hard to predict the actual situation of transgene introgression and spread in wild or weedy rice populations with the fitness estimation, based only on the limited data generated from sporadic experimental studies. Therefore, it will be very useful to combine experimental data collected from field studies and computer

simulation (modeling) to predict the likelihood of transgene introgression and spread in wild or weedy rice populations that have incorporated the transgenes through pollen-mediated gene flow under different environmental conditions.

## 8. General conclusions

Transgene escape from GM rice to its wild or weedy populations through gene flow may pose potential ecological consequences, due to the unique characteristics of transgenes that are genetically modified. Cultivated rice and its close wild relatives (including weedy rice) containing the AA-genome exist sympatrically in many parts of the world, and these species also have considerable overlaps in their flowering time. Therefore, crop-to-wild or crop-to-weedy transgene flow in rice will happen at different frequencies depending on the species and populations involved, and should not be neglected. The extent of ecological consequences from such transgene escape essentially depends on whether the transgenes will normally express in wild rice relatives, and how the transgenes will change the fitness of wild or weedy populations that have picked up the transgene, under a favorable or unfavorable selection pressure. Our preliminary research data showed that the insect-resistance *Bt* transgene can express normally in hybrids crossed with common wild rice, and higher amount of *Bt* toxin was detected in some individuals of the crop-wild hybrids and F<sub>2</sub> progeny. This indicates that the likelihood of transgene to express normally or exceedingly in wild relatives of rice would be high. The measurement of fitness change in crop-wild and crop-weedy hybrids as well as their later generations is very useful for determining the potential ecological consequences although it is challenging, owing to many factors that are involved to influence the fitness of crop-wild and crop-weedy hybrids with transgenes. So far, little is known in terms of fitness change that associates with transgene introgression into wild or weedy rice populations. Further assessment on ecological consequences caused by transgene escape from GM rice to its wild and weedy rice is necessary. Particularly, knowledge of the evolutionary potential of a transgene in wild populations under diverse environmental conditions should be generated to help a more appropriate assessment of such consequences, which requires more studies on fitness change after transgene introgression. Better understanding of ecological consequences associated with transgene escape will facilitate the safe and sustainable cultivation of GM rice in agro-ecosystems for food production.

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