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Achieving successful deployment of *Bt* rice

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An additional billion mouths will need feeding within the next decade. In spite of the extensive research and development that has been invested in *Bt* rice (rice transformed with insecticidal genes from *Bacillus thuringiensis*) and the success of *Bt* maize and *Bt* cotton, *Bt* rice is not grown commercially. *Bt* rice has the potential to eliminate yield losses caused by lepidopteran pests, estimated at 2–10% of Asia's annual rice yield of 523 million tons. Cultivation of *Bt* rice should also lead to substantial reductions in the use of broad-spectrum chemical insecticides, with benefits for farmers' health, environmental quality and biological control of other rice pests. The major challenges are developing appropriate resistance management strategies and resolving trade policy impediments.

The global population is steadily growing while the amount of arable land is steadily decreasing. Thus, it is essential that sustainable strategies be implemented to use agricultural resources efficiently to yield an abundant healthy diet. Experience to date with genetically engineered crops has shown that this technology can make substantial contributions towards this goal. Among the first transgenic crops approved for release were *Bt* maize and *Bt* cotton, which contain genes encoding insecticidal proteins from the bacterium *Bacillus thuringiensis*. These crops have been readily adopted by farmers, have resulted in increased yields and reductions in insecticide applications, and have been sustainable when used with resistance management programmes [1]. However, continuing controversies surrounding the risks and benefits of this novel technology have prevented its benefits from reaching consumers in many parts of the world.

Rice is the target crop for many improvement programmes because it is the staple diet for nearly two billion people worldwide and the major food for over half of those living in Asia [2]. At the 2003 International Congress of Plant Pathology (Christchurch, New Zealand), Zhangliang Chen (President of the China Agricultural University) announced that China was feeding 22% of the world's population using only 7% of the available land* and that their productivity is currently dependent on heavy chemical inputs. In 2002, rice production in China reached 177 million tons, of which 3.1 million tons was exported, producing revenue of US$578 million (http://www.irri.org/science/ricestat/index.asp). These numbers represent only a fraction of what might be available if rice plants were not subject to insect attack. Many rice varieties have been transformed with genes encoding various *Bt* crystal (Cry) proteins and have been shown to be resistant to one or more lepidopteran pests of rice (Table 1), the most important of which are the yellow stem borer (*Scirpophaga incertulas*), the striped stem borer (*Chilo suppressalis*) and several species of leaf-folders (*Marasmia* spp. and *Cnaphalocrocis medinalis*) [3]. Field trials of *Bt* rice commenced in China in 1998 [4–6] and in India in 2001 (S.K. Raina, pers. commun.) (Table 2), but no *Bt* rice or other transgenic rice varieties have yet been released for commercialization. Here, we review the potential benefits of *Bt* rice, biosafety issues, approaches to resistance management and the regulatory issues that continue to delay the release of this technology to farmers.

Benefits of *Bt* rice

The use of *Bt* cotton has resulted in substantial decreases in insecticide use in developed and developing countries, and, in some cases, also in increases in yield and profitability [1,7]. For example, farmers growing *Bt* cotton in India gained a 70% reduction in insecticide applied, a saving of US$30 per hectare in insecticide costs and a revolutionary yield increase of 80–87% [7]. In a survey conducted in China in 1999, the average number of pesticide applications by farmers using *Bt* cotton was 6.6 per crop, compared with 19.8 for farmers growing conventional cotton, and the proportion of farmers reporting pesticide poisoning symptoms was 4.7% compared with 22% [8]. Hopefully, anticipated downstream public health improvements will continue to be monitored in *Bt*-cotton-growing areas, documenting the longer-term

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Table 1. *Bt* rice lines with resistance to lepidopteran pests[a]

Cultivar	Promoter	Gene	Refs
KMD1[b], KMD2[b]	Ubiquitin	*cry1Ab*	[4,57]
Elite Eyi 105, Bengal	Ubiquitin	*cry1Ac*	[58]
IR64, Pusa Basmati-1, Karnal Local	Ubiquitin	*cry1Ac*	[59]
Basmati 370	Ubiquitin	*cry1Ab*, *cry1Ac*	[60]
IR58	CaMV 35S	*cry1Ab*	[61]
IR72, IR-64, CBII, Taipei-309, IR68899B, MH-63–63, IR51500-AC11, Vaideh-1, IRRI-npt	CaMV 35S, Actin-I, pith tissue-specific, PEPC	*cry1Ab*	[62]
Tarom Molaii	PEPC	*cry1Ab*	[63]
IR64	Ubiquitin	*cry1Ac*	[64]
Vaidehi, TCA-48	CaMV 35S	*cry1Ab*	[65]
Basmati-370, M-7	CaMV 35S	*cry2A*	[66]
Kaybonnet, Nipponbare, Zhong8215, 93VA, ZAU16, 91RM, T8340, Pin92-528, T90502	Ubiquitin	*cry1Ab*, *cry 1Ac*	[67]
Taipei-309	CaMV 35S	*cry1Ab*	[68]
Basmati 370	Pollen-specific, ubiquitin, PEPC	*cry1Ab*	[69]
Ariete, Senia	Ubiquitin	*cry1B*	[70]
Ariete	Maize proteinase inhibitor	*cry1B*	[71]
CMS restorer Minghui 63[b], Shanyou 63[b]	Actin-1	*cry1Ab/cry1Ac* hybrid gene	[5]

Abbreviations: CaMV, cauliflower mosaic virus; PEPC, phosphoenolpyruvate carboxylase.

[a] Data modified from Ref. [72].

[b] Lines have been field tested in China.

benefits of reductions in the use of broad-spectrum insecticides.

Decreases in insecticide use and increases in yield and profitability resulting from *Bt* rice, although still substantial, will be less dramatic than those for *Bt* cotton. Rice stem borers and leaf-folders cause less yield loss than do lepidopteran pests of cotton and are the targets of fewer insecticide applications. It is challenging to quantify yield losses in rice because of the large and variable area over which the crop is grown. In the most rigorous study to date, Serge Savary *et al.* [9] found that total yield losses caused by weeds, diseases and insects ranged from 24% to 41% depending on location and production situation. Among insect pests, stem borers (the principal target of *Bt* rice) caused the greatest yield loss, estimated at 2.3% [9]. Typically, yield losses caused by stem borers have been estimated at 5–10% [3]. In China, losses caused by rice stem borers, in spite of the use of insecticides, were estimated as 3.1% of the national rice yield, equivalent to a monetary loss of 6.45 billion renminbi (RMB) (US$780 million) [10]. The cost of chemical control of stem borers was estimated as RMB2.85 billion, with ecological and health costs of RMB1.45 billion to RMB2.85 billion and RMB0.3 billion, respectively [10]. Total pesticide costs account for less than 3% of the production costs of Asian rice farmers but the number of applications varies greatly [11]. For example, farmers in Zhejiang Province (China) (Figure 1) apply 20 times as much active ingredient as those in Central Luzon (The Philippines). The relatively small proportion of production costs attributable to pesticides is in part a result of the relatively low cost of pesticides and of a decline in insecticide use in some countries where integrated pest management (IPM) training programmes have been implemented. In IPM courses, farmers learn that most insecticide applications are unnecessary because yield losses are generally overestimated and that insecticides disrupt biological control of pests by predators and parasitoids [12]. Early-season sprays directed at leaf-folders have been particularly targeted by insecticide reduction programmes because rice plants can readily compensate for defoliation that occurs at the vegetative stage [13].

In relation to the size of Asia's rice harvest of 523 million tons (http://www.irri.org/science/ricestat/index.asp), the estimated 2–10% yield loss attributable to stem borers and leaf-folders across the region represents an enormous amount of rice. In addition, insecticide reductions that will result from the introduction of *Bt* rice will confer

Table 2. Milestones in the development of *Bt* rice

1981	First *cry* gene cloned and sequenced	[73]
1984–1999	Inception of The Rockefeller Foundation's International Rice Biotechnology Program	
1985	Transformation of plants with *cry* genes	[74–77]
1986	First field tests of plants transformed with *cry* genes in the USA and France	
1988	First field trials of *Bt* cotton in the USA	[78]
1993	First published report of *japonica* rice transformed with *cry* gene	[79]
1994	Opening of containment greenhouse at International Rice Research Institute, The Philippines, for growth of transgenic plants	
1995	First published report of *indica* rice transformed with *cry* gene	[61]
1996	First growing season by farmers of *Bt* maize, *Bt* cotton and *Bt* potato	
1997	First growing season by farmers of *Bt* cotton in China	[8]
1997	Field trials of *Bt* cotton begin in India	[7]
1998–1999	Field tests of *Bt* rice begin in China	[4–6]
1999	First published report of rice transformed with two *cry* genes	[55]
2001	Field tests of *Bt* rice begin in India	S.K. Raina, pers. commun.
2002	First growing season by farmers of *Bt* cotton in India	[7]

Figure 1. Intensive rice production over the past 40 years has seen the widespread use of pesticides by farmers, particularly insecticides, perhaps one of the most damaging environmental consequences. Insecticide use has exploded because of both supply and demand factors [11]. Photograph courtesy of Gongyin Ye.

important benefits to environmental quality and the health of farmers and consumers. Particularly, extensive pesticide run off from rice fields pollutes not only ground water but also environments far away from rice paddies. Furthermore, because many pesticides are not degraded before the rice is eaten, they pose potential risks for rice consumers. For these reasons, the benefits of *Bt* rice might be far greater than the benefits of *Bt* cotton. Rice currently imported into Canada, for example, can contain the following pesticide levels: 25.0 ppm HCN, 5.0 ppm quinclorac, 2.0 ppm methoxychlor, 0.5 ppm Naled, 0.05 ppm bentazon, 0.1 ppm other pesticides (http://www.hc-sc.gc.ca/food-aliment/friia-raaii/food_drugs-aliments_drogues/act-loi/pdf/e_c-tables.pdf). A global goal should be to engender regulations that would see these contaminants removed from the rice supply and replaced by Cry proteins as expeditiously as possible.

With respect to transgenic crops in general, much is made of hypothetical unintended effects – not all of these are necessarily negative. A valuable unintended benefit observed in *Bt* maize is a reduction in levels of mycotoxins [14], some of which are known carcinogens. In maize, the single determinant of mycotoxin in the grain is grain damage, which is caused almost exclusively by insect feeding. Rice grains can be contaminated with mycotoxin-producing fungi during growth [15]. It is possible that stem borer larvae that feed on developing panicles increase mycotoxin contamination. *Bt* rice might also reduce the spread and propagation of fungi in storage if the grains are resistant to feeding by lepidopteran pests of stored grain, such as the Angoumois grain moth (*Sitotroga cerealella*) or the rice moth (*Corcyra cephalonica*).

Biosafety concerns

Outcrossing

Asia was the origin of the genus *Oryza*. Two common wild rice species in Asia, *Oryza nivara* and *Oryza rufipogon*, have the same AA genome as cultivated rice, *Oryza sativa*, and are known to hybridize with cultivars under field conditions [16,17]. In addition, there are many weedy rice types in Asia derived from *O. rufipogon*, *O. nivara*, *O. sativa* or hybrids of cultivars and wild rice [18]. Not all rice crops flower synchronously with neighbouring wild rice populations and, when they do, the outcrossing rate is generally low. For example, one study in Hunan Province (China) [19] found a maximum of 3% outcrossing to *O. rufipogon*. Cultivated rice is primarily self-pollinated and outcrossing among cultivars occurs at a low rate. The outcrossing rate between transgenic and non-transgenic cultivars in a field test in Spain ranged from 0.05 to 0.53% [20]. Nonetheless, given the vast area over which rice is cultivated and wild and weedy rices occur, transgenes will almost certainly escape into non-transgenic plants. Novel technologies under development that can restrict pollen fertilization and seed germination might reduce the rate of outcrossing when transgenic pollen does happen to encounter non-transgenic flowers [21]. The extent to which transgenes will persist and spread in wild and weedy rices and in non-transgenic cultivars and the possible consequences of outcrossing will need to be assessed on a case-by-case basis.

Dozens of improved rice cultivars containing single major genes conferring resistance to insects such as the brown planthopper and rice gall midge or to diseases such as rice blast or rice tungro virus have been widely grown in Asia since the 1970s [2]. Traditional rice cultivars and wild rice species have served as the sources of these resistance genes, which have been transferred to modern cultivars by conventional breeding. There are no known cases in which wild or weedy rice populations have become more aggressive as a result of outcrossing of resistance genes from cultivars, although we are not aware of any studies that have examined this question. Pre-release studies on the impact of transgene outcrossing might be appropriate in some areas for transgenes that confer traits that are not found in traditional cultivars or wild rice species, such as high levels of resistance to stem borers (as conferred by *Bt cry* genes) or to abiotic stresses such as drought or salinity. Many rice-growing areas of Asia, such as central China, do not harbour populations of *O. rufipogon* or *O. nivara*, whereas in others, such as the Mekong Delta of Vietnam, one or both of these species are abundant [22]. Surveys in the Mekong Delta indicate that stem borer and leaf-folder populations on *O. rufipogon* and weedy rices are generally low, suggesting that outcrossing of *Bt* genes to these plants will not strongly affect their distribution or abundance (M.B. Cohen *et al.*, unpublished).

Impact on biodiversity

An outstanding feature of *Bt* crops is that *cry* toxins have little or no effect on non-target organisms [23]. The introduction of conventional insecticides to Asian rice production in the 1970s had a devastating impact on arthropod predators and parasitoids of rice pests, resulting in vast outbreaks of the brown planthopper (*Nilaparvata lugens*) [12]. Insecticides also drastically reduced the populations of fish and crabs in rice fields, which are harvested for food. By contrast, results from laboratory and field studies with *Bt* maize, *Bt* cotton and *Bt* potato indicate that these crops are generally not toxic to beneficial arthropods and non-target organisms [24]. Laboratory and field studies to date with *Bt* rice provide a similarly positive picture, although monitoring in larger field plots will be essential when cultivation of *Bt* rice on a larger scale is permitted. In small-scale field trials in China, population densities of five common spiders were similar in plots of *Bt* and non-*Bt* rice [25]. Brown planthoppers reared on *Bt* rice were found to ingest *Bt* toxins but were not toxic when fed to the most important predator of planthoppers, *Cyrtorhinus lividipennis* [26]. In a field experiment in The Philippines, *Bt* sprays were used to simulate a major effect of *Bt* rice, the removal of foliage-feeding caterpillars, and to quantify the impact on the arthropod community [27]. No disruptions in the biological control of non-target herbivores were found. It is likely that the introduction of *Bt* rice will enhance biological control in rice fields if farmers reduce insecticide applications directed against lepidopteran pests. Gongyin Ye *et al.* [6] noted that, although no planthopper outbreaks occurred in their plots of *Bt* rice (which received no insecticide applications), outbreaks did occur in nearby insecticide-sprayed fields of non-*Bt* rice.

Food safety

More than 100 years of science supports the use of *B. thuringiensis* as a biological pesticide system [28]. The US Environmental Protection Agency has found no evidence that *Bt* crops currently registered are toxic or allergenic to humans [23]. Studies have reported an absence of acute, subchronic and chronic oral toxicity to mammals associated with *Bt* microbial pesticides, which also contain Cry proteins [29,30]. Investigations of the digestive fate of recombinant DNA and proteins have found that the amount of intact transgenic DNA and proteins in the digestive tract is minimal to zero [31]. Chickens fed YieldGard® Corn Borer maize event MON 810 (Monsanto, http://www.monsanto.com) were analysed and none of the extracted chicken breast muscle samples contained any detectable transgenic DNA or Cry1Ab protein [32]. In another study, pigs fed transgenic *Bt* maize were slaughtered, and no recombinant or maize-specific DNA was detectable in the tissue samples [33]. A study of cows fed *Bt* maize found only short DNA fragments (<200 bp) present in the blood lymphocytes; in all other organs, plant DNAs were not detected [34].

Three recent studies have examined the food safety of *Bt* rice. *Bt* rice flour containing the *cry1Ab* gene was fed to test rats in a 90-day feeding trial and no toxic effects were found at dosages of up to 64 g per kg body weight [35]. Parental and *Bt* rice were compared for their major nutritional components and physicochemical properties. There were no significant differences in major nutritional components (crude protein, crude lipid, free amino acids, total ash and mineral elements) between the primary transgenic rice KMD and the wild-type Xiushui 11, or between the recurrent parent Jiazao 935 and new transgenic line Huachi B6, which was bred using KMD as the insect-resistant donor. Although a small amount of Cry1Ab protein was detected in raw rice, no transgenic protein was detected in the cooked rice [36]. In much more extensive feeding trials currently under way with *Bt* rice and rice expressing a plant haemagglutinin known to be toxic to mammals, animals fed the *Bt* rice variety KMD1 presented microindicators that were essentially identical to those observed in animals fed the nontransgenic parental variety Xiushui 11 rice (Q.Y. Shu *et al.*, unpublished).

Resistance management

Insect adaptation to conventional insecticides and crop cultivars has been a pervasive and costly problem in pest management [37]. As yet, there have been no measurable increases in the frequency of resistance in pest populations exposed to *Bt* crops in the field, but many insect species have been shown to evolve resistance to Cry proteins under laboratory conditions. The absence of pest resistance to *Bt* crops is probably due in part to the implementation of resistance management strategies in countries where the crops have been released on a large scale and in part to other factors such as fitness costs of alleles conferring resistance [38]. In the USA and Australia, farmers who grow *Bt* crops are required to maintain refuges (fields or rows of non-*Bt* cultivars) corresponding to 5–50% of the area planted with *Bt* cultivars. Refuges maintain sufficient numbers of wild-type susceptible insects in the population, reducing the likelihood that insects carrying mutations for resistance will mate with each other and produce homozygous resistant progeny. *Bt* cotton farmers in China are not required to plant refuges. Instead, it has been argued that alternative host plants of the principal pest, *Heliothis armigera*, serve as refuges [39]. In most cases (*Bt* maize with resistance to rootworms being the exception), the USA also requires that *Bt* cultivars contain a high dose of toxin. This is defined as a dose that results in functional recessiveness of pest alleles that confer resistance.

The biology of rice stem borers and the socioeconomics of rice cultivation present a challenge to the design and implementation of resistance management strategies for *Bt* rice. Because most rice in Asia is grown on small farms and extension services have limited reach and influence, it will be difficult in most areas to establish and enforce a policy of mandatory refuges. In addition, with minor exceptions, the yellow stem borer and the striped stem borer feed only on rice, so refuges will not be provided by alternative host plants [40]. Governments can, however, implement policies that might result in the maintenance of adequate refuges [41]. First, governments can require that *Bt* rice cultivars have two toxins, both produced at a high dose and chosen such that a single insect mutation is unlikely to confer resistance to both [42,43]. Such two-toxin cultivars would require smaller refuges than would single-toxin cultivars [42,44]. Biochemical studies have identified several appropriate toxin combinations for rice stem borers (e.g. Cry1Ab with Cry2A [45,46]). Because four different *cry* genes have been transformed into rice lines (*cry1Ab*, *cry1Ac*, *cry1Ba* and *cry2A*), a concerted effort should be made to cross these lines to produce at least two-toxin rice, if not three- or four-toxin rice (Table 1). Second, governments can restrict the number of popular cultivars that are released in *Bt* form and maintain seed supplies of non-*Bt* cultivars. Farmers who choose to grow non-*Bt* cultivars would in effect be providing a refuge for neighbouring fields planted with *Bt* cultivars. The non-*Bt* farmers would benefit from a possible area-wide decrease in pest populations similar to the decrease in pink bollworm populations in *Bt*-cotton-growing areas of the southwestern USA [47].

Regulation and public policy

Research into GM crops is advancing rapidly in many Asian countries, with over 100 private and publicly funded laboratories working on improving crop quality and yields in China alone [48]. China has acknowledged and emphasized the importance of biotechnology in improving agricultural crops since the late 1980s [8] (http://yaleglobal.yale.edu/display.article?id=2526). There have been several successful field trials of *Bt* rice in China [4,5,49] but commercialization of *Bt* rice has been delayed by government officials. Following China's admittance into the World Trade Organization in December 2001, China implemented strict regulations regarding the management of biotechnology that have effectively delayed the potential release of commercial *Bt* rice. One factor currently delaying the release of transgenic rice by many rice-producing countries is the *prima facie* regulations (http://www.wto.org/english/res_e/booksp_e/analytic_index_e/sps_03_e.htm) implemented by some rice-importing countries, such as those of the European Union, that restrict the import of GM food products. There is thus a direct relationship between trade policies in rice-importing countries and continued insecticide consumption amongst rice producers. An initial impediment to the development and release of transgenic crops in Asia, the lack of national biosafety regulations, is being overcome as more and more countries implement biosafety frameworks. A second probable factor is consumer acceptance. Rice is the staple food for most Asians and occupies a unique position in many cultures. Surveys indicate that most consumers are not aware of transgenic crops [50], presenting both a challenge and an opportunity to governments, research institutions and companies developing *Bt* rice. The successful introductions of *Bt* cotton in China and India, and of *Bt* maize in the Philippines [51] have demonstrated that these countries have functioning regulatory systems for the review and release of transgenic crops, and will provide incentive for the eventual release of *Bt* rice. The release of *Bt* rice would also be further expedited if other transgenic rice cultivars are released first. It is possible that 'golden rice' (with enhanced levels of provitamin A) [52] and *Xa-21* rice (containing a gene for resistance to bacterial blight from the wild species *Oryza longistaminata*) [53] will be the first transgenic varieties approved for release.

Conclusion

The criteria for the successful deployment of *Bt* rice in Asia include safety, sustainability and expeditiousness. Continued research and development and evolution of the regulatory environment are necessary if these criteria are to be met (Box 1). Resistance management is the major technical challenge for *Bt* rice. Given the monophagous nature of rice stem borers and the limited ability to influence farmer behaviour in most areas, how can adequate refuges be maintained? Development and field testing of rice containing two appropriate toxin genes, both expressed at a high dose, continues to be a research priority [42,54]. Only two reports of *Bt* rice lines pyramiding two appropriate toxin genes (*cry1Ac* and *cry2A*) have been published [55,56]. There is also a need for extensionists to produce educational materials and programmes for farmers to communicate the benefits of and best agronomic practices for *Bt* rice. Although *Bt* crops have an excellent record of environmental safety and compatibility with biological control, monitoring of impacts on rice agro-ecosystems will be essential when large-scale plantings of *Bt* rice are approved.

Box 1. Future research and regulatory needs for successful deployment of *Bt* rice

- Resistance management strategies compatible with rice production systems in Asia.
- High-dose *Bt* rice cultivars with two toxin genes, each of which binds to a different receptor.
- Resistance monitoring programmes.
- Case-by-case evaluation of the possible impact of *cry* gene outcrossing to wild and weedy rices.
- Studies of the fate of Cry toxins in tropical flooded soils.
- Programmes for monitoring the impact of large-scale *Bt* rice plantings on biological control.
- Educational materials for farmers and consumers.
- Trade policies authorizing the importation of transgenic rice.

The negative consequences of further delays in releasing *Bt* rice include continued overuse of broad-spectrum insecticides and their severe effects on farmer health, biological pest control and environmental quality. Given the disastrous state of rice paddy water quality caused by disruptive chemical sprays, the gains in biodiversity alone justify the implementation of extensive field trials. Our own preliminary small-scale field trials have led to encouraging observations of increased amphibian and fish wildlife in rice paddy water where *Bt* rice is grown and insecticide applications eliminated. In addition, there is an ongoing need to increase rice yields to assure continued food security in Asia.

It is known that *Bt* rice has the potential to increase yields, to decrease pesticide applications and hence to improve groundwater quality, and possibly also to reduce mycotoxin levels. The substantial potential benefits offered by *Bt* rice, particularly in light of the success of *Bt* cotton and *Bt* maize, should be powerful motivators for further development of improved *Bt* rice lines and accelerated approval of the release of *Bt* rice to farmers.

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