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China's Ministry of Agriculture granted safety certificates in August 2009 to two transgenic varieties of rice and one transgenic maize for commercialization (Table 1). The safety certificates granted for these three strains are valid from August 2009 to August 2014. During this time, the approved rice and maize varieties are to be commercially planted in the Hubei and Shandong provinces, respectively.

As the first transgenic food crop approved in China, many people are showing their concerns over commercializing these varieties in China. However, little information is available to the public regarding both the pest resistant rice Huahui No.1 and the *Bt* Shanyou 63. This review is an attempt to clarify the development, molecular characterization, welfare potentials and biosafety issues regarding these two genetically modified rice lines.

Development

The transgenic event TT51-1 was generated by the Huazhong Agriculture University through direct DNA transfer via co-transformation of two separate plasmids: pFHBT1 harboring *cry*1Ab/Ac gene and pGL2RC7 harboring *hph* selective marker gene.[1] TT51-1 became the predecessor of Huahui No.1, which harbors a hybrid *cry*1Ab/Ac gene consisting of 1,344 bp encoding the N-terminus of *cry*1A(b) and 486 bp encoding C-terminus of *cry*1A(c). The transgenes are regulated by the rice *actin* 1 promoter and the *nopaline synthase* (NOS) gene terminator.

Controlled field trials for the transgenic lines were performed in 1999 and 2000. Moreover, two environmental releases were performed during 2001 and 2002 and two preproduction field trials were carried out during 2003 and 2004. In 2004, the developer submitted the application for release of Huahui No.1 to the Ministry of Agriculture (MoA). It was only in 2009 when the developer finally received the biosafety certificate for commercialization.

Regulatory checks were carried out during the period from 2004–2008. China MoA ordered safety tests including molecular characterization and risk assessment of environmental and food biosafety. Some of the tests were conducted by qualified third party laboratories. The results ensured that there were no food and environmental risks under the Ministry's guidelines (see below). After the final biosafety assessment from the National Biosafety Committee (NBC), Huahui No.1 and a related *Bt* Shanyou 63 were issued biosafety certificates for commercial production in Central China Hubei province in August 2009.

NBC was established under the MoA as the decision making body. It is composed of officials from the MoA and scientists representing variety of disciplines including agronomy, biotechnology, plant protection, animal science, microbiology, environmental protection and toxicology. The committee meets 2–3 times a year to evaluate the biosafety applications related to experimental research, field trials, environmental release and commercialization of agricultural GMOs. Based on the results of biosafety assessments of the studied GMOs, the committee passes its recommendations of approval or disapproval to the MoA officials.

Nevertheless, in addition to a safety certificate for commercializing a certain

Table 1. List of approval to the applications for the biosafety certificates of agricultural genetically modified organisms

No.	Coding of approval	Organization of applicant	Tittle of application	Validity
41	Agri-GMO biosafety certificate (2009) No. 072	Huazhong agricultural university	Biosafety certificates for pest-resistant rice restorer line Huahui No. 1 containing the transgenes cry1Ab/cry1Ac to be produced in Hubei Province, China	From August 17, 2009 to August 17, 2014
42	Agri-GMO biosafety certificate (2009) No. 073	Huazhong agricultural university	Biosafety certificates for the pest-resistant rice hybrid Bt Shanyou63 containing the transgenes cry1Ab/cry1Ac to be produced in Hubei Province, China	From August 17, 2009 to August 17, 2014
43	Agri-GMO biosafety certificate (2009) No. 074	Biotechnology research institute, Chinese academy of agricultural sciences	Biosafety certificates for the genetically modified maize line BVLA430101 containing the transgene phytase to be produced in Shandong Province	From August 17, 2009 to August 17, 2014

Source: http://www.stee.agri.gov.cn/biosafety/spxx/P020091127591594596689.pdf

Table 2. Characterization of the insert DNA sequence and the flanking sequences in TT51-1 (or Bt Shanyou 63)

No.	Position	Length	Homology	Genes
1	1–673	673	Rice genome	5' flanking genome DNA
2	674–880	207	pSZ72 428-222 (plus/minus)	
3	881–3171	2291	Pactin1—Cry1Ab/Ac-Tnos	Incomplete Cry1Ab/Ac cassette
4	3174–3240	67	pSZ72 67-133	
5	3290–3388	99	pSZ72 257-355	
6	3388–3937	549	pSZ72 1913-2462	Containing 83 bp of Amp gene
7	3972–7513	3542	Pactin1—Cry1Ab/Ac-Tnos	Intact Cry1Ab/Ac cassette
8	7516–9236	1721	pSZ72 67-1787	Containing 653 bp of Amp gene
9	9238–9287	50	Hph gene	Incomplete Hph gene
10	9365–10536	1172	Rice genome	3' flanking genome DNA

product, GM seed developers must register their GM seed variety at the provincial agricultural department as required by the People's Republic of China Seed Law. This process would take another 2–3 years.

Molecular Characterization

According to the Southern blot analysis of the transgenic rice plants, Huahui No.1 contained a single copy of the transgene and proved to be genetically stable throughout the tested generations.[1,2] Wu et al. certified the integrity of the insert and characterized the flanking sequences of the transgenic event TT51-1.[1-3] Their results revealed that two copies of *cry*1Ab/Ac cassettes and one copy of incomplete *hph* gene were co-integrated at the same locus, and partial deletions occurred to the *Actin1* promoter and the selectable *hph* gene. The joint presence of the *cry*1Ab/Ac cassettes and the *hph* gene at the same locus indicated that during pre-integration phase, the truncated plasmid molecule pFHBT1 was ligated with a fragment of *hph* gene that was derived from the selective plasmid pGL2RC7. They were integrated in the same locus giving rise to the rearranged transgenes. The 5'-end of transgenes was integrated into a scattered repeated sequence motif in the TT51-1 event. In addition to the trait gene *cry*1Ab/Ac and the selectable *hph* gene, the transgenes of TT51-1 included two fragments of *amp*^{r} gene; 83 bp and 653 bp in length, respectively. This suggests that during the process of transformation, the backbone of the transformation plasmid was integrated into the host genome as well. The complete sequence of event TT51-1 was submitted to GenBank under the Accession Number EU880444.

Based on the flanking sequences of 5' and 3' plasmid-genome junctions, the qualitative and quantitative event-specific detection method of TT51-1 event were established (**Table 2**).[3]

Agronomic Benefits

Huahui No.1 and *Bt* Shanyou 63 varieties showed strong resistance against the striped stem borer (*Chilo suppressalis*), the yellow stem borer (*Scirpophaga incertulas*) and the leaf folder (*Cnaphalocrocis medinalis*). Both lines demonstrated excellent agronomic performance as well.[2] In 1999, *Bt* hybrid Shanyou 63 yielded 28.9% more than the control in the presence of natural and induced infestation of leaf rollers and yellow stem borers. In the field trail conducted in Wuhan in 2002 and 2003, no pesticides were sprayed in the field.[2] An increase of 6–9% in yield was recorded over the conventional rice varieties. Moreover, a reduction of 80% in the application of pesticides was achieved using the *Bt* rice in later production trials.

Bt rice has the potential to reduce, if not to eliminate, yield losses caused by lepidopteran pests. Losses, estimated at 2–10% of Asia's annual rice yield, can be alleviated since *Bt* rice reduces the spread and propagation of fungi in storage if the grains are resistant to feeding by lepidopteran pests of stored grain, such as the common pest Angoumois grain moth (*Sitotroga cerealella*) and the rice moth (*Corcyra cephalonica*).[5]

According to Huang et al. (2005), *Bt* rice offers the potential to generate benefits of US $4 billion annually from an average yield increase of 8% and an 80% decrease in insecticides usage, equivalent to 17 kg per hectare on China's major staple food crop that occupies 30 million hectares every year.[4]

Risk Assessment

The food safety assessment was performed on toxicity, allergenicity and nutrition aspects based on the substantial principles. More than 100 years of research supports the use of *Bacillus thuringiensis* as a biological pesticide.[6] It has long been known that *Bt* microbial pesticides, which also contain Cry proteins, are safe to mammals as well as to the environment.[6-8] *Bt* corn has been grown and consumed for more than 13 years without any reported acute, subchronic or chronic toxicity. At a 90 day feeding test with *Bt* rice flour at a dosage of 64 g Kg^{-1} body weight, *Bt*, NPTII and HPT proteins had no toxic effects on rats.[9] Toxicological effect of *Bt* rice on the development of silkworm (*Bombyx mori*) larvae showed a noticeable loss of body weight, but did not show any lethality.[10] Although less than 2.5 μg Cry1Ab protein per gram of rice was detected in raw rice flour, no transgenic protein was detected in the cooked rice since the *Bt* protein was denatured during the process of cooking.[11,12]

The environmental biosafety was assessed by considering the effects of *Bt* transgene and the transgenic plants on non-target organisms, on biodiversity, on gene flow and on the development of resistance in the target organisms. 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.[13] In small-scale field trials in China, population densities of five common spiders were similar in plots of *Bt* and non-*Bt* rice.[14] 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*.[15] 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. The review panel determined that *Bt* rice have no marked negative effects on the arthropod community in paddy fields and there is no significant difference between *Bt* and non-*Bt* rice on species and family composition and their abundance and indices of community structure of arthropods. However, Lu et al. considered that *Bt* gene may confer environmentally selective advantages and it may quickly spread through introgression of crop-weed or crop-wild hybrids in the weedy or wild populations.[16] He suggested that some strategies of risk management should be taken to control gene flow from *Bt* rice to wild and weedy rice.[17]

According to the results of the risk assessment, the NBC reported that the Huahui No.1 and *Bt* Shanyou 63 are safe both for food consumption as well as for the environment.

Concluding Remarks

Bt rice has the potential to increase yield, decrease pesticide applications, improve groundwater quality and possibly reduce mycotoxin levels in the stored grains. The substantial potential benefits offered by *Bt* rice is a powerful motivator for further development of improved *Bt* rice lines and for accelerating the process of approving the release of transgenic crops to the consumers. After issuing the biosafety certificate, the *Bt* rice is awaiting to pass various regional trials and to be granted the approval of seed production before releasing to the market.

Approval of *Bt* rice in China is a landmark decision since it has serious impacts and implications for GM crops around the world. Issues ranging from the concerns about food and environmental biosafety, intellectual property rights and impact on farmers' income are debated through the media. During the Chinese National People's Congress in March 2010, these debates became particularly hot. Given the importance of rice as a staple crop, a broad discussion among all strands of society is needed to evaluate the impact and importance of GM crops for China.

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