Rice Science, 2014, 21(5): 271−281

DOI: 10.1016/S1672-6308(13)60197-3

Transgene Flow from Glufosinate-Resistant Rice to Improved and Weedy Rice in China

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Abstract: The development of transgenic rice with novel traits in China can increase rice productivity, but transgene flow to improved or weedy rice has become a major concern. We aimed to evaluate the potential maximum frequencies of transgene flow from glufosinate-resistant rice to improved rice cultivars and weedy rice. Treatments were arranged in randomized complete blocks with three replicates. Experiments were conducted between 2009 and 2010 at the Center for Environmental Safety Supervision and Inspection for Genetically Modified Plants, China National Rice Research Institute, Hangzhou, China. Glufosinate-resistant japonica rice 99-1 was the pollen donor. The pollen recipients were two inbred japonica rice (Chunjiang 016 and Xiushui 09), two inbred indica rice (Zhongzu 14 and Zhongzao 22), two indica hybrid rice (Zhongzheyou 1 and Guodao 1), and one weedy indica rice (Taizhou weedy rice). The offspring of recipients were planted in the field and sprayed with a commercial dose of glufosinate. Leaf tissues of survivors were analyzed by polymerase chain reaction to detect the presence of the transgene. The frequency of gene flow ranged from 0 to 0.488%. In 2009, the order of gene flow frequency was as follows: weedy rice > Chunjiang 016 > Xiushui 09 and Zhongzu 14 > Guodao 1, Zhongzheyou 1 and Zhongzao 22. Gene flow frequencies were generally higher in 2009 than in 2010, but did not differ significantly among rice materials. Gene flow frequency was the highest in weedy rice followed by the inbred japonica rice. The risk of gene flow differed significantly between years and year-to-year variance could mask risk differences among pollen recipients. Gene flow was generally lesser in taller pollen recipients than in shorter ones, but plant height only accounted for about 30% of variation in gene flow. When flowering synchrony was maximized, as in this study, low frequencies of gene flow occurred from herbicide-resistant japonica rice to other cultivars and weedy rice. Averaged across years, the risk of gene flow to weedy rice was higher than that of improved rice and hybrids. Greater resources must be dedicated to the management of remnant weedy rice in fields planted with herbicide-resistant rice, and to prevent the evolution of resistant weedy rice populations.
Key words: gene flow; hybrid rice; japonica rice; indica rice; transgenic rice; weedy rice; glufosinate-resistance

Rice (*Oryza sativa* L.) is the principal food for approximately half of the world's population (Mohanty, 2013). Approximately 80% of rice produced globally is consumed in Asia (USDA-FAS, 2013), and the top rice-consuming countries are China (31%), India (21%), Indonesia (9%) and Bangladesh (7%) (Mohanty, 2013). With the increase of the world's population, the demand for rice has outpaced the production levels. Land and water resources for rice production are limited and declining. Thus, rice productivity can only be increased by improving yield through a combination of various improved crop traits, either by classical plant breeding or by genetic engineering. Of the two approaches, the latter has gained momentum in recent decades because of rapid advances in plant genomics, bioinformatics and biotechnology. In fact, biotechnology is the fastest-growing technology that is being adopted for crops (James, 2012).

The first batch of traits that have been genetically engineered are protection traits, such as resistances to insect pests (Gao et al, 2011; Yang et al, 2011; Akhtar et al, 2013; Qi et al, 2013), pathogens (Ma et al, 2011; Takakura et al, 2012; Verma et al, 2012; Helliwell et al, 2013; Shah et al, 2013; Shimizu et al, 2013), and herbicides (Oard et al, 1996; Olofsdotter et al, 2000;

Received: 5 November 2013; **Accepted:** 15 April 2014
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Inui et al, 2001; Hu et al, 2009; Jung et al, 2010; Wakasa et al, 2012; Li et al, 2013). Stacking multiple transgenic crop protection traits by classical plant breeding has been accomplished (Wei et al, 2008). Transgenic rice genotypes with improved traits, including tolerance to abiotic stress, improved nutrient content, improved nitrogen uptake and utilization, and high yield (Lu and Snow, 2005; Lu and Yang, 2009; Kajala et al, 2011; Kurai et al, 2011; Caemmerer et al, 2012; Fang et al, 2013), have been produced or are currently being developed. Among the abovementioned traits, only insect-tolerant trait in rice expressing the *Bt* gene has been approved for commercialization (James, 2011), although many of these traits have been successfully transferred to different rice varieties and tested in the fields (Oard et al, 1996; Jiang et al, 2000; Huang et al, 2002; Jia and Peng, 2002; Li, 2004; Lu and Yang, 2009).

The first herbicide-resistant transgenic rice genotypes were developed at approximately the same period in the 1990s by scientists at the Louisiana State University, USA (Oard et al, 1996) and the China National Rice Research Institute (CNRRI, Hangzhou, China) (Hu et al, 2000). Resistance to the non-selective glufosinate herbicide was developed by inserting the *bar* gene (de Block et al, 1987; Thompson et al, 1987) from *Streptomyces hygroscopicus* into the plant genome. Herbicide-resistant rice provides breeders in CNRRI with an efficient method of segregating hybrid seeds from self-pollinated ones (Hu et al, 2000). Transgenic rice with glufosinate or glyphosate resistance has not been released commercially, despite being an exceptional tool for weed control in rice, because of general consumer rejection of genetically modified rice. Nevertheless, non-transgenic, herbicide-resistant (Clearfield®) rice is now widely adopted in Americas and Italy, and is commercially released in Malaysia (Sudiantu et al, 2013).

Given the expected production of superior cultivars with the protection traits, high yield and improved nutrition qualities, transgenic rice may be commercially released and accepted by consumers. Adoption of transgenic food crops, specifically insect-resistant crops, has continued to increase globally because the technology drastically reduces the load of harmful pesticides in the environment and human exposure to pesticides (James, 2012). Despite the apparent benefits of transgenic crops, such as rice with the protection traits or improved grain quality, the expectation of its widespread adoption has raised concerns on the possible impact of gene escape on food safety, agroecology (escape to weedy and wild relatives), and conservation of native landraces (OECD, 1999; Lu and Snow, 2005).

Rice is predominantly self-pollinated, but < 1% natural cross-pollination with its weedy relatives and higher outcrossing with the wild relatives is well documented (Gealy, 2005; Shivrain et al, 2007). Gene flow frequency within inbred rice varieties is similar with that within weedy rice (Rong et al, 2004). However, gene flow between hybrid rice and weedy rice in the southern USA reaches a high percentage of 1.26% on average, whereas the highest average gene flow between inbred rice and weedy rice is only 0.26% (Shivrain et al, 2009). Outcrossing rate with wild rice (*O. rufipogon* L.) reaches 2.94% (Song et al, 2002, 2003; Chen et al, 2004). Considerably higher gene flow frequencies (> 50%) were reported in different male-sterile lines than in other lines (Jia et al, 2007; Yuan et al, 2007). Genetic compatibility, breeding behavior and environmental factors cause a wide fluctuation in gene flow frequencies within the *Oryza* complex (Shivrain et al, 2008, 2009; Niruntrayakul et al, 2009; Song et al, 2009; Zuo et al, 2011; Pusadee et al, 2013). Introgression of the protection traits or abiotic stress tolerance traits into weedy relatives has raised concern on the possible occurrence of more serious weedy rice problems. Similarly, transgene introgression into native landraces and non-transgenic varieties compromise the germplasm resources or the purity of food supply meant for consumers who are averse to genetically modified food products. Preventing the escape of transgenes or the segregation of crop products is a daunting challenge, as demonstrated by the case of Liberty Link® rice 601 (Vermif, 2006) and similar incidents in other transgenic crops.

Rice production areas in China can be divided into the following six ecological areas: double crop rice (indica) in south China (approximately 18%); single and double crop rice (indica and japonica) in central China (approximately 68%); single crop rice (indica and japonica) in southwest China (approximately 8%); single crop rice (indica and japonica) in north China (approximately 3%); single crop rice (japonica) in northeast China (approximately 3%); and single crop rice (japonica) in northwest China (approximately 0.5%) (Cheng and Li, 2007). In general, indica rice is predominantly planted in south China, japonica rice in north China, and a mix of indica and japonica varieties in central China (Ministry of Agriculture,