Gene flow from genetically modified rice to their weedy and wild relatives and its environmental consequences

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Rice is one of the most important food crops in the world. Nearly half of the world population consume rice as their staple food (FAO, 2004). With the increasing of world population, the need to provide more food supplies including rice is obvious. Biotechnology is expected to play major roles in the improvement of crop productivity and quality. Unlike transgenic maize, eventhough many research have been done to improve rice performance by genetic engineering, transgenic rice has not been released or commercialized. Among those research to improve rice performance are the atempt to introduce biotic and abiotic stress tolerant traits, herbicide tolerant trait and beta-carotin biosynthetic pathway. Currently, several genetically modified rice cultivars have been and being tested on limited field trials.

Undoubtedly, biotechnology will benefit agriculture and thus providing enough food source to keep up with the ever-increasing needs in the future. However, the release and utilization of such technology in agriculture is still arising concerns about the impact to the environment. Therefore the possibility of the transgene escape to the environment needs to be analized. For example, whether the application of such technology can create superweed that resulted in the environment problems. On the other hand, cultivated rice may acquire genes for weediness from pollen-mediated gene flow of weedy or wild rice occurring inside or near cultivated rice fields, which leads to persistence and invasiveness of the cultivated rice, although the chance is low.

This article discuss the possibility of the occurence gene flow from the application of genetically modified rice to surounding rice plants including its wild relatives and weed, and the requirements or precautions needed to be done to prevent gene flow.

Gene Flow

Gene flow through polination is a natural process occurs in nature in which wind or insect play roles in the process. Although, undoubtedly gene flow may occur naturally, there are conditions for it to occur: 1. Reproductive compatibility, 2. Sympatry, 3. Flowering synchrony, and 4. Wheather condition. Reproductive compatibility had a major influence on the cross ability of transgenic rice and weedy rice (Song et al, 2009). Actually, beside through pollen dispersal, gene flow from a cultivated rice varieties could also occurs through seed dispersal and vegetative propagation (Lu and Yang, 2009).

This article focuses on gene flow which occurs due to pollen dispersal from cultivated rice varieties to their weedy and wild relatives. Cultivated rice varieties would include genetically modified rice.

Gene pool of cultivated rice and their wild relatives

Initial study to determine which weedy or wild relatives has the ability to perform hybridization (sexual compatibility) with the cultivated lines tested resulted in fertile progeny is important. To answer that question, understanding gene pool of a cultivated line and its weedy or wild relatives is necessary (Figure 1).

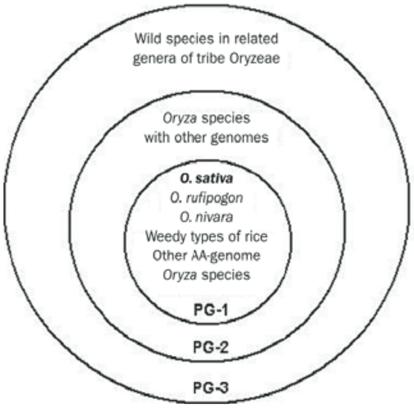

Figure 1. The gene pool of Asian cultivated rice (*Oryza sativa* L.**)**. The primary gene pool (PG-1) of Asian cultivated rice comprises its weedy types (*Oryza sativa f. spontanea*), ancestral wild species (*Oryza rufipogon* and *Oryza nivara*), and other AA-genome Oryza species. The secondary gene pool (PG-2) comprises other non-AA-genome wild species in the genus Oryza, and the tertiary gene pool (PG-3) includes species in other genera within the tribe Oryzeae in the grass family (Poaceae) (Source: Lu et al., 2004).

Gene pool for the Asian rice cultivar can be categorized into primary, secondary and thertiary gene pool. The rice *Oryza sativa* which are originated from South and South-East Asia are now being cultivated around the world and many of the cultivars have been genetically engineered. This facts indicate that the posibility of the transfer of the transgene into their weedy or wild relatives are important to be studied.

Weedy rice relative with AA genome that is widely found in rice growing area around the worl and capable of hybrydization with cultivated rice are those known as the brown rice (*O. sativa* f. Spontanea). Meanwhile, six wild rice species with AA genome which are found around the world are also identified and have the potential to hybridize cultivated rice. This include the perenial rice *O. rufipogon* and the annual rice *O. nivara* from Asia, the perenial rice *O. longistaminata* and the annual rice *O. arthii* from Africa, the perenial rice *O. glumaepatula* from South America, and the annual rice *O. meridionalis* from Northern Australia and New Guinea. All of those rice taxa can hybridize their cultivated relatives and among themselves (Vaughan 1994; Vaughan et al., 2003). Additionally, genetically, there are varieties of brown rice in which in some cases they can hybridize with wild relatives (*O. rufipogon*) (Vaughan et al., 2003).

Oryza species with different genom types have obvious reproductive isolation resulted in sexual incompatibility which inhibit hybridization with cultivated, or weedy and wild relative with different genome types. Hybridization among species from different genera within Oryzeae are difficult to perform even by using modern techniques, such as embryo rescue.

Because of its reproductive structures, rice tends to self pollinate and therefore are considered low risk in term of gene flow through hybridization (Stewart et al., 2003). However, if the transgene from the genetically modified rice induces stronger fitness, the transgene would quickly spread among the weedy and wild relatives (Ellstrand et al., 2003).

Gene flow from cultivated rice to cultivated rice

In general, cultivated rice with self pollinating tendency will have lower chance of cross pollination with the surounding rice plants (less than 1%). Study in Italy showed that the occurence of gene flow from pollen of genetically modified rice (herbicide tolerant rice) to the surounding non-transgenic rice plants are very small between 0.05-0.53% (Messeguer et al., 2001). The finding is consistent with the recomended distance to perform isolation and to maintain line purity of a cultivated rice grown in a nursery. In the US, rice grown for sertification purposes to be sold to farmers has to be isolated from other rice varieties within 6 meters (Gealy et al., 2003).

Gene flow from cultivated rice to weedy rice

Cultivated and weedy rice are self pollinating and have short pollen life span, therefore the possibility of gene flow is very low. However, studies on the possibility has been done. The study of gene flow of a transgene from a genetically engineered rice Nam29/TR18 carrying *bar* gene to 13 accessions of weedy rice from Asia and America have been performed. The bar gene expressing herbicide (ammonium glufosinate/Basta) tolerant trait. The rice cultivar Nam29/TR18 is a transgenic rice from the cross between rice cultivars Nam29 and transgenic rice TR18. The experiment design for the gene flow study is as described in Figure 2.

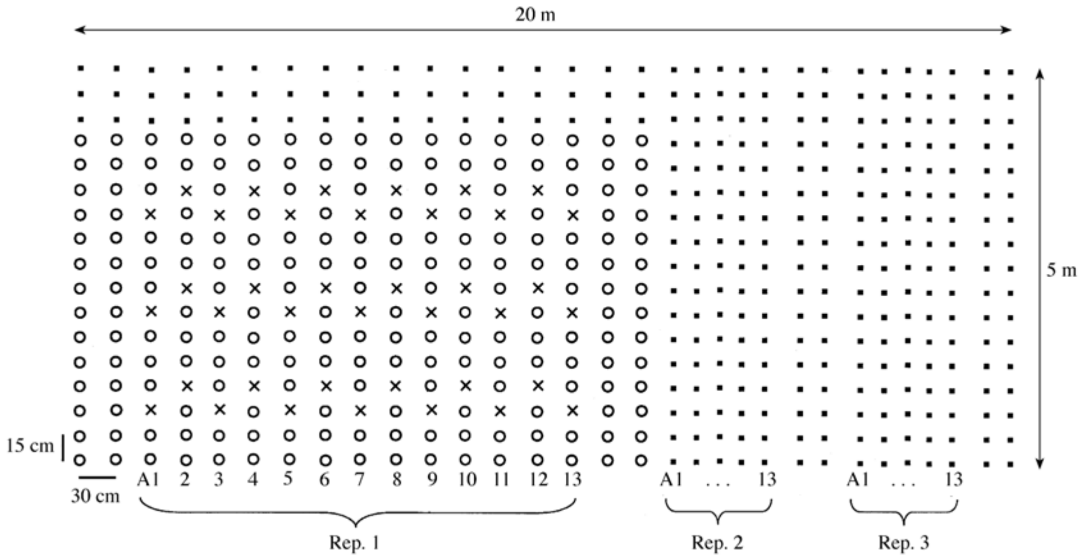

Figure 2. Field experimental design of gene flow from genetically modified rice to its weedy relative. Circle indicates Nam29/TR18 rice lines, cross indicates weedy rice. The experiment was performed with 3 replicates. Each replicates consists of 13 blocks (A1-A13). In each column 35 rice plant were planted, including 8 accessions of the weedy rice chosen randomely among the 13 total accessions. In each column, after 3 transgenic rice, a weedy rice was planted. To make sure enough pollen donor on the outer border between replicates 6-7 column of rice cultivar Nam29/TR18 were planted (Chen et al., 2004).

To identify the hybrid cross between Nam29/TR18 and weedy rice, seeds from the weedy rice were germinated and leaves number 3 and 4 were sprayed with Basta. Seedlings tolerant to Basta were considered as hybrid and were subsequently analyzed using PCR to determin the presence of the *bar* gene. Seedlings which were positive with *bar* gene proved that gene flow from the genetically modified rice to its weedy relative occured (Figure 3).

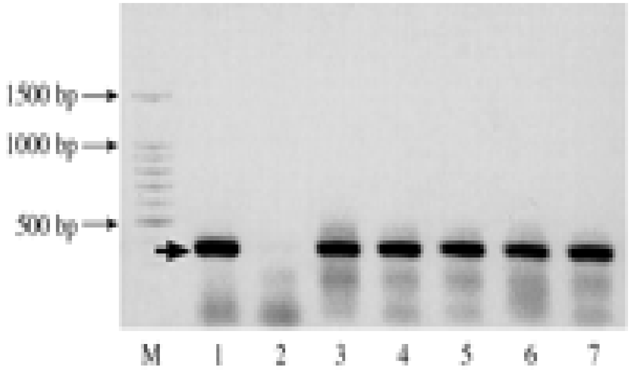

Figure 3. Detection of the bar gene from the germinated weedy plantlet after treatment with the herbicide Basta. M: DNA ladders; lane1: Nam29/TR18; lane 2: weedy rice; lane 3-7: Basta tolerant weedy rice (Chen e al., 2004)

The frequency of gene flow was determined by calculating seeds that were survived after ten days of basta treatment divided by the total number of germinating seeds. The final frequency of the gene flow was determined by comparing the data obtained from the basta spray to those of the PCR data. Results showed that the percentage of the gene flow to occur from the transgenic rice to its weedy relative was between 0.011-0.046% (Chen et al, 2004). The chance is very low and even some weedy accessions did not show any indication of gene flow from the cultivated rice line. Results from similar experiments in Italy and Spain by Messeguer et al (2001), showed that the occurence of gene flow from transgenic rice to its weedy relative was between 0.01-0.53%. In China and the Europe similar experiments showed that the frequency was between 0-0.06 (Chen et al., 2004; Messeguer et al., 2004).

Similar study was also conducted by Song et al (2009). Homozygous transgenic japonica rice (Y0003 and 99-t) carrying one copy of the *bar* gene were used as the materials. Five accession of weedy rice were used (WR1, WR2, WR3, WR4 and WR5). Results showed that gene flow from the transgenic rice to their weedy relatives can occur although in a low possibility (Figure 4).

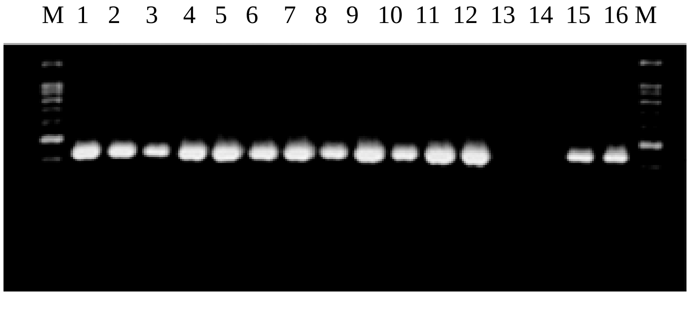

Figure 4. Amplification of *bar* gene. M: DNA ladders; lane 1-3: F1 WR2x Y0003; lane 4-6: F1 WR2x 99-t; lane 7-9,15: Y0003; lane 10-12,16: 99-t; lane 13-14:WR2 (Source: Song et al., 2009)

Mainly the low frequency of gene flow from the cultivated rice to its weedy relative is due to the difference of flowering time and plant height. Heading date of the weedy rice are 4 – 22 days earlier or latter than the transgenic rice Nam29/TR18. They are also 10-60 cm taller than the transgenic rice (Chen et al, 2004). On the contrary, Zuo et al (2011) found that most of the weedy rice biotypes (WRLN001, WRLN004, WRJS043, WRAH007, WRYN004, WRJS001, WRJS015, WRJS017, WRJS020, WRAH002, WRHB002, WRZJ003 and WRGD011) differed significantly from the transgenic rice Y0003 in plant height, panicle height, panicle length and the number of fertile tillers, as well as in flowering time. Y0003 was taller than all of the weedy rice biotypes except WRGD011 and WRYN004. The TWRGD011 and WRYN004 which were the tallest and the WRNM001 and WRHL001 which were the shortest weedy biotypes did not out-cross at all with Y0003. The reason that none of the shortest weedy rice biotypes (WRNM001 and WRHL001) formed outcrosses may be because they are too short for their stigma to catch floating pollen grains. All the weedy biotypes taller than Y0003 also failed to hybridize. Since the height of WRAH002, WRZJ003 and WRAH007 biotypes were similar to Y0003, had the highest out-crossing rates. And, weedy biotypes that slightly shorter than Y0003 had the highest out-crossing rate, which might be attributed to the falling trajectory of dispersed Y0003 pollen grains due to gravity.

Gene flow from cultivated rice to weedy rice

Several studies detect gene flow from cultivated rice to their wild species, in which the rate of the occurence was higher than that of to the weedy relatives (Song et al., 2003, Cheng et al., 2004). The study of gene flow from a cultivated rice cultivar Minghui 63 to its wild relative *O. rufipogon* has been performed. Experimental was designed in a 5x5 m2 plot with 2 replicates (Figure 5). Co-dominan simple sequence repeats (SSRs) was used as molecular marker to accurately identify crosses between Menghui and *O. rufipogon*. Primer pairs SSR RM44 (forward: ACGGG CAATCCGAACAACC;reverse:TCGGGA AAACCACCCTACC) was used to amplify polimorphic allele from the two species which are easily visualized by 3-4% agarose gel electroporesis. DNA from leaves of germinating plants of Minghui-63, *O. rufipogon* and the hybrid from the cross were collected for SSR analysis. The observation of the SSR analysis indicated that *O. rufipogon* showed consistent bands migrated faster (F), Menghui -63 showed bands moved slower (S), while the hybrid between the two species showed both bands migrated faster (F) and slower (S) (Figure 6).

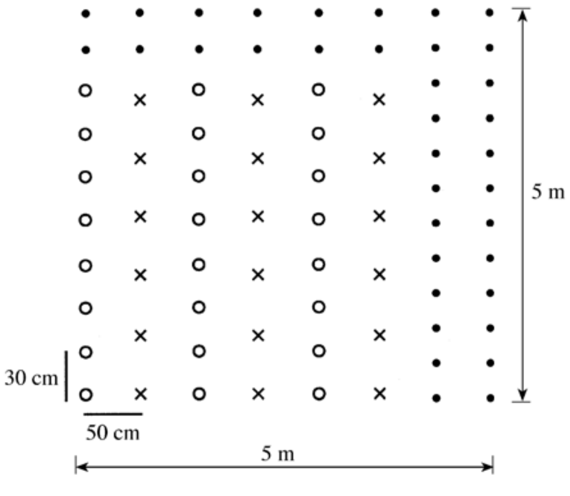

Figure 5. Field trial design for gene flow study from cultivated rice to its wild relative. Circle indicates rice cultivar Minghui-63, cross indicates the wild relative *O.rufipogon* (Chen et al., 2004).

The frequency of gene flow was calculated by dividing the number of seedlings with SSR FS heterozygot with the total number of seedlings observerd. Results from the experiment showed the frequency of gene flow from cultivated rice to its wild relatives was between 1.21-2.49% (Chen et al., 2004).

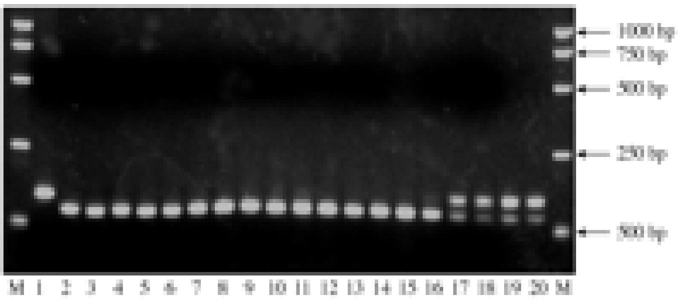

Figure 6. PCR amplification using the RM44 SSR specific primers. Lane M: DNA ladders; lane 1: Minghui 63; lane 2: *O. rufipogon*; lane 3-16: selfing of *O. rufipogon*; lane 17-20: hybrid between Minghui 63 dan *O. rufipogon* (Chen et al., 2004)

Lu et al (2004) stated that the maximum frequency of the occurence of gene flow from cultivated species to their wild relatives was less than 3%, while 95% of the hybrid was obtained from the wild relatives planted within 30 meters of the cultivated rice. The maximum distance within which gene flow could be detected in the wild rice was 110 meters. The study concluded that the low frequency of gene flow from cultivated rice to its wild relative can occur if *O. rufipogon* was planted within 50-100 m of the cultivated rice.

Consequences of transgene escape from genetically modified rice to its weedy and wild relatives

Several studies have detected evident of gene flow from cultivated rice to weedy rice and wild rice. Weedy and wild rice can be easily found through out Asia. Weedy rice can addapt in the diverse ecosystem and grow along side cultivated rice. Paddy field is the major habitat for weedy rice. Weedy rice competes with cultivated rice for sunlight, water and nutrients, therefore, decreasing the quality and yield of the cultivated rice, especially in the paddy field using direct seeding (Zhang et al., 2006). Weedy rice can substantially reduce cultivated rice production (Baki et al., 2000). Results showed that the decrease of rice production due to weedy rice using direct seeding was 22.1% (Lee et al, 1987). Weedy rice is difficult to control because seeds tend to spread before harvesting and accumulated in soil as seed bank. The seeds of the weedy rice can also be accidently, due to carelessness, collected by the farmers during harvest and then planted together with the cultivated seed. Competition with cultivated rice and contamination of cultivated seed with weedy seeds are serious problems for rice farmers.

The frequency of gene flow from cultivated rice to its weedy (0.011-0.046%) and to its wild relatives (1.21-2.94%) are different. The variability of the frequency are depending on the genetic distance between the varieties used in the experiment, which influence the reproductive compatibility for successful hybridization. The lower the genetics distance between the cultivated rice and weedy or wild relatives, the closer their compatibility. Reproductive compatibility between species with flowering synchrony was suggested to be the main factor controlling the success of natural hybridization between them. The weedy rice stigmas morphological characteristics (i.e. the number of feathers, the length of feathers and the condition that making the pollen easier to agglutinate) affected the number of transgenic pollen grains adhering. Furthermore, pollen grains of cultivated plants sticking to, and germinating on, the stigmas, and subsequent growth in the styles and entry into the ovules of weedy rice, had a major influence on the crossing success between cultivated rice and weedy rice (Zuo et al., 2011). Environmental conditions such as wind strength, air humidity and temperature are other important factors influence pollen –mediated gene flow (Lu dan Yang, 2009). Thus, the important factors affected the success of gene flow are reproductive compatibility, flowering synchrony, plant height, morphology of the stigmas, pollen grains conditions, and wheather condition such as wind strength, air humidity and temperature.

Gene flow resulting from hybridization between cultivated plants and their weedy and wild species can be either beneficial or harmful depending on the types of genes transferred. If the transgene that transmited to weedy and wild species can enhance the fitness of weedy rice or wild rice with favorable traits such as pest resistance, drought tolerance, and enhanced growth ability, this transgenes are unwanted transgenes. And followed by gene flow these transgenes would persist to and quickly spread in the populations of weedy and wild rice through introgression. Under natural selection, the individuals of weedy and wild rice that have picked up those transgene will out-compete other individuals without the transgene (Lu and Snow, 2005; Lu, 2008). This process may enhance their invasiveness, causing different degrees of weed problems and enabling the wild populations to quickly expand to new territories. Traits that may influence invasiveness include fertility, vegetative vigor, tolerance to a wide range of environmental conditions, and the quality and dispersal range of viable material.

On the contrary, if the transgene reduces the fitness of weedy rice or wild rice, 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 Genetically Modified (GM) crop, leading to the extinction of local populations by the so-called swarm effect (Ellstrand and Elam, 1993). If the new genes incorporated into weedy or wild plant give the plant neither special advantage nor help it to survive, it is unlikely to really make much of a difference in the plant population. For example if the transgene are intended to improve nutrition content, then the trasgene will have neutral or even negative impact to the fitness of the weedy or wild relatives.

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 on the knowledge of potential adverse or harmful effects from a transgenic plant and their probability to occur. (Lu dan Yang, 2009).

Since weedy rice is already a problem in more than 50 countries in Asia, Africa and Latin America in reducing rice yield and quality, Chen *et al.* (2004) recommended that transgenic rice with herbicide tolerance genes or with genes that can significantly enhance the ecological fitness of weedy rice, should not be released in regions where weedy rice is already a serious problem. Or, special precautions needs to be implemented on the application of such rice to the environment.

The risk of gene flow to adjacent non-transgenic fields could be minimized by establishing isolation zones between transgenic rice varieties and weedy rice or wild rice with sufficient space or with some tall plants to act as pollen traps. Another approach is to insert the new gene into the chloroplast of the cell instead of the nucleus. This works because pollen only carries genes from the nucleus and does not carry a chloroplast or chloroplast genes. GM crop pollen may be able to fertilize wild plants, but the new gene does not go with it.

As one of the center of origin of rice, Indonesia has to pay close attention on the possibility of the occurence of gene flow from transgenic rice to weedy or wild rice. The possibility that such gene flow may increase the survivability and reproductive advantage of the weedy and wild relatives needs to be assessed. It is necessary for any release of transgenic rice (or plants) to the environment to undergo risk analysis and assessment. The analysis will include the biology of host plant (natural range, reproductive method, viability, weediness characteristics), novel trait (s) introduced, assessment on biodiversity including the impact on non-target organisms, gene flow and its consequences, weediness potential/ invasiness. And if the product of transgenic plants is for food then food safety analysis will have to be be included. In Indonesia, regulations concerning the release of genetically modified organisms to the environment implemented by the Goverment Decree (Peraturan Pemerintah/PP) No. 21/2005 on the Biosafety of Genetically Enggineered Product.

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