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Pollen-Mediated Gene Flow between Glufosinate Ammonium-Tolerant GM and Non-GM Rice

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Abstract

To assess the risk of genetically modified (GM) rice on the agricultural ecosystem, agronomic characteristics, pollen longevity and outcrossing rate between GM (Iksan 483 and Milyang 204) and non-GM (their wild types and female parents) varieties were investigated using the *bar* gene as a tracer marker in paddy field. The agronomic characteristics of two GM rice were similar to their female-parents (non-GM rice) except heading date and 1,000 grain weight of Iksan 483, and they did not show a difference by the introgression of the *bar* gene as the genetic traits of rice varieties. Pollen viability was more than 90% just after shedding, and it was rapidly decreased below 50% at 5 minutes after shedding both GM and non-GM varieties. The Pollen longevity was lost after 30 minutes of anthesis. When the distance of gene flow from GM to non-GM rice detected to 6 m from the edge of GM rice plant, the maximum distance of pollen dispersal was 4.5m and 3.9m in Iksan 483 and Milyang 204, respectively, and that was increased in order of west, south, east, and north to the dominant wind direction, west-south. Mean outcrossing rate was very low as 0.003 and 0.001% within 1.5 m from the edge of Iksan483 and Milyang 204, and the GM hybrids by the pollen dispersal did not detected over 4.5 m from the edge of GM rice plant. The results may help to establish the strategy which reduce the risk of pollen-mediated gene flow between GM and non-GM rice.

Introduction

Rice (*Oryza sativa* L.) is one of major crops as a staple food source of the world's population. Biotechnology tools in rice are utilized to achieve the resistance to diseases and insects, tolerance to herbicide, and tolerance to biotic and abiotic stresses. In middle of the 1990's, the rapid advances in biotechnology had led to the commercial production of genetically modified (GM) crops such as tomato, soybean and maize, and untill recently, GM crops have been developed in more than 100 plant species including maize, wheat, soybean, tomato, potato, cotton, rice. Herbicide and insect tolerant GM crops among them are grown on a commercial scale (Mohan Babu et al. 2003). In 2005, the global area of approved GM crops was 90 million hectares, and GM crops were grown by approximately 8.5 million farmers in 21 countries (James 2005).

GM rice variety has not been commercialized worldwide yet, but in recent, there has reported the development of commercially important GM rice such as Bt rice (Tu et al. 2000), provitamin A-enriched rice (Datta 2003; Ye et al. 2000), and iron-enriched rice (Vasconcelos et al. 2003). The GM rice varieties with improved nutraceutical values or cultural convenience will be cultivated to the rice farmers for commercial production in developing countries of Asia and Africa in the next few years. Especially, the herbicide tolerant GM rice will be more widely adopted for the rice farmers, because weed control is one of the farmer's biggest challenges in direct seeding cultivation. However, herbicide tolerant GM rice has to assess the risks associated with gene flow against super weediness potential before environmental release. In commercialized GM crops, the most gene flow is known to transfer by pollen dispersal (Kareiva et al. 1994). Especially, the gene flow can be a serious weediness in GM crops with cross-pollination system such as corn, oilseed rape, sunflower, cotton and others (Meier-Bethke and Schiemann 2003; Thompson et al. 1999; Arias and Rieseberg 1994).

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As well as, in rice with self pollination system, gene flow from GM rice to its weedy and wild relatives has been predicted the serious weediness potential. Since the 1980's, the hybrids between cultivated rice and wild rice with AA genome containing red rice are fertile, and are considered to be weed problems in various parts of the world (Langevin et al. 1990, Chen et al. 2004). The gene flow between rice and red rice is possible at far distance (Messeguer et al. 2001, 2003), and the outcrossing rate is very high (Langevin et al. 1990, Oard et al. 2000). Accordingly, the prediction of gene flow in rice field will help to reduce the serious weediness potential caused by herbicide tolerant GM rice.

The objective of this study was to assess the risk of GM rice on the agronomic ecosystem. agronomic characteristics, pollen longevity and outcrossing rate between GM (Iksan483 and Milyang 204) and non-GM (their wild types and female parents) varieties were investigated using the *bar* gene as a tracer marker in paddy field.

Materials and Methods

Plant Materials and Cultivation

Two glufosinate ammonium-tolerant GM events, Iksan 483 and Milyang 204 were selected from the progenies crossed between cultivated varieties (female parents, Sindonjinbyeo and Junambyeo) and the T_1 plants (male parents) originated from Anjungbyeo and Donjinbyeo (T_0) which transformed with the *bar* gene as a tracer marker. Iksan 483 and Milyang 204, herbicide resistant GM lines contained stably incorporated copy of the *bar* gene, and expressed tolerance to 0.3% Bastar® (Bayer Crop Science Korea Co.). The field trial for agronomic characteristics, pollen longevity and outcrossing rate between GM and non-GM rice were conducted from 2004 to 2005 at biosafety rice field in Korea. Seeds of each variety were sown in seedling nursery box containing commercial soil (Punongsangto Co., Korea) on 1th May, and seedlings grown in field nursery till 5th June when they reached at 4-5 leaf stages. The transplanting of Milyang 204 and Iksan 483 were used the tolerant seedlings to 0.3% Bastar® (Bayer Crop Science Korea Co.) at 3th to 4th leaf stage before transplanting. Soil preparation, irrigation and fertilization of paddy field were managed by the standard cultivation method of Honam Agricultural Research Institute, NICS, RDA, Korea. Nitrogen, phosphorous (P_2O_5) and potassium (K_2O) were applied at 110, 70 and 80 kg/ha, respectively. Nitrogen fertilizer was split-applied with urea as basal 50%, 25% at tillering stage and 25% at panicle initiation stage. All field trials were conducted using the randomized complete block design with three replications.

Growth Characteristics of GM Rice

To compare growth characteristics of GM and non-GM rice varieties, the 30-day-old seedlings were transplanted in four rows (30 plants/row) per plot with 3 plants per hill. Growth characteristics were sampled in ten hills per replication for culm length, panicle length and panicle number per hill, in three hills per replication for grain filling rate and 1,000 grain weight, and in each replication for heading date.

Pollen Longevity Between GM and Non-GM Rice

For comparison of the pollen viability and longevity, field-grown GM and non-GM plants of seven varieties were transferred into individual pots (1 hill per pot, 5 replications) at 60 days after transplanting, and were grown to the flowering stage in greenhouse. On the day of flowering, shed pollens of GM (Iksan 483 and Milyang 204) and non-GM rice varieties (Sindongjinbyeo, Anjungbyeo, Junambyeo and Donjinbyeo) were collected on hole slide glass at anthesis time, and were supplemented with one drop of pollen germination medium after keeping for 0, 3, 5, 10, 15 and 30 minute from shedding time. The corrected pollen grains after shedding were held under 32±2℃ of temperature, 65±3% of humidity and 16,280±1,268 lux of sunlight in green house. The slide grasses without cover glass were layed on the filter paper saturated with distilled water in Petri dish, and were germinated for 1.5 to 2 hours in 30℃ incubator. Germinated pollen grains were fitted with one drop of acetocarmin (1%), and were counted under microscope. Pollen germination medium was contained 600 mg/L $Ca(NO_3)\cdot {}_2O$, 100 mg/L H_3BO_3 and 10% Sucrose, and was adjusted with pH 5.5.

Pollen Dispersal and Outcrossing Rate from GM to Non-GM Rice

The frequency of gene flow by pollen dispersal was evaluated by a square design with pollen recipient populations in east, west, south and north. The herbicide tolerant GM rice varieties (Milyang 204 and Iksan 483) as pollen donor were transplanted with 3×3m square size (10 row spacing 30×15cm, 20 plants per row) in center of each plot. Non-GM rice varieties (Dongjinbyeo to Milyang 204, Anjungbyeo to Iksan 483) as pollen recipient were transplanted with 3×6m size (20 row spacing 30×15cm, 20 plants per row) in east, west, south, and north of each plot. At 50 days after heading, samples of seeds were harvested five panicles per hill in four directions of recipient populations. The harvested seeds were sown in field on 30th April, 2005, and the seedlings at the 3 to 4 leaf stage were treated with 0.3% commercial herbicide Bastar®. After 3 weeks, each surviving seedling was transferred into individual pots, and were retreated with 0.3% Bastar®. All survival seedlings were extracted DNA from young leaves, and introgression of *bar* gene was conformed by electrophoresis of PCR products (Lee et al. 2006). Outcrossing rate through pollen dispersal from GM to non-GM rice was estimated the number of survival plant to a total germinated plant with spacing 1.5 m of each direction.

Results

Growth Characteristics of GM Rice

For comparing the growth characteristics between GM (Iksan 483 and Milyang 204) and non-GM rice, agronomic characteristics were investigated in paddy field (Table 1). Days to heading ranged 105~110, and heading date of Iksan 483 was earlier 3~4 days than tested varieties except Anjungbyeo and Milyang 204. Heading date of Milyang 204 was similar to non-GM rice varieties. Culm length ranged 65.2~90.6 cm. Culm length of Iksan 483 was similar to Shindongjinbyeo and Anjungbyeo, and that of Milyang 204, to Junambyeo. Milyang 204 showed the shortest panicle length, but it was highest in the number of panicle per plant among the tested varieties. Filled grain rate ranged 96~86%, and 1,000 grain weight ranged 21.4~27.6 g. Thus some agronomic characteristics showed a varietal difference, but the values were distributed in the range of cultivated rice varieties. In two GM rice, Iksan 483 and Milyang 204, most agronomic characteristics were similar to those of Anjungbyeo and Junambyeo, respectively. Accordingly, agronomic characteristics of two GM rice were belong to the ranges of cultivated rice varieties, and did not show a difference by the introgression of the *bar* gene.

Pollen Longevity Between GM and Non-GM Rice

For comparing pollen longevity between GM (Iksan 483 and Milyang 204) and non-GM rice (their female parents, Shindongjinbyeo and Junambyeo), pollen germination after shedding of pollen grains was investigated in green house (Table 2). The percentage of pollen germination in the fresh pollens of four varieties ranged 90.2~95.1% just after shedding. The percentages of pollen germination in two GM rice, Iksan 483 and Milyang 204 were 37.0% and 48.1%, 19.8% and 27.8%, and 6.6% and 11.2% at 5, 7 and 10 minutes after shedding, respectively. The viability of

Table 1. Growth characteristics in herbicide tolerant GM (Iksan 483, Milyang 204) and non-GM rice (their wild types and female parents).

Variety	Days to Heading	Culm length (cm)	Panicle length (cm)	No. of Panicle/plant	Filled Grain (%)	1,000 grain weight
Iksan 483^{GM}	106c^{z}	73.4c	20.5bc	11.6ab	91.4abc	21.4c
Sindongjinbyeo^{FP}	109b	74.9c	23.0b	11.2b	96.0a	27.6a
Anjungbyeo^{WT}	105c	73.3c	21.6bc	11.9ab	86.7c	23.4bc
Milyang204^{GM}	108bc	69.5cd	19.1c	13.9a	90.6abc	21.6bc
Junambyeo^{FP}	110b	65.2d	21.7bc	13.0ab	86.9bc	22.5bc
Dongjinbyeo^{WT}	110b	90.6b	21.9bc	12.1ab	95.1ab	23.8bc

^{GM}Genetically modified variety, ^{WT}Wild type, ^{FP}Female parent.
^{z}Data followed by the same letter in each column were not significantly different at the 5% level based on Duncan's multiple range test.

Table 2. Pollen viability according to time after pollen shedding between GM (Iksan 483, Milyang204) and non-GM rice (their female parents)

Time after shedding (min.)^{y}	Pollen germination (%)			
	Iksan 483^{GM}	Sindongjinbyeo^{FP}	Milyang 204^{GM}	Junambyeo^{FP}
0	90.2a^{z}	91.3a	95.1a	93.5a
3	80.5a	85.7a	80.9a	76.7a
5	37.0b	32.0b	48.1b	42.8b
7	18.9c	14.2c	27.8c	28.9c
10	6.6d**	2.3d	11.2d	9.9d
15	2.4de**	0.3d	2.0de	1.6de
30	0.0e	0.0d	0.0e	0.0d

^{GM}Genetically modified variety, ^{FP}Female parent.
^{y}Experiment was conducted under 32±2℃ temperature, 65±3% humidity and 16,280±1,268 lux sunlight in greenhouse. Pollen germination was controlled with 30±1℃ temperature, 90±2% humidity, and dark condition.
^{z}Data followed by the same letter in each column were not significantly different at the 5% level based on Duncan's multiple range test. **Significant to female parent at $P<0.01$.

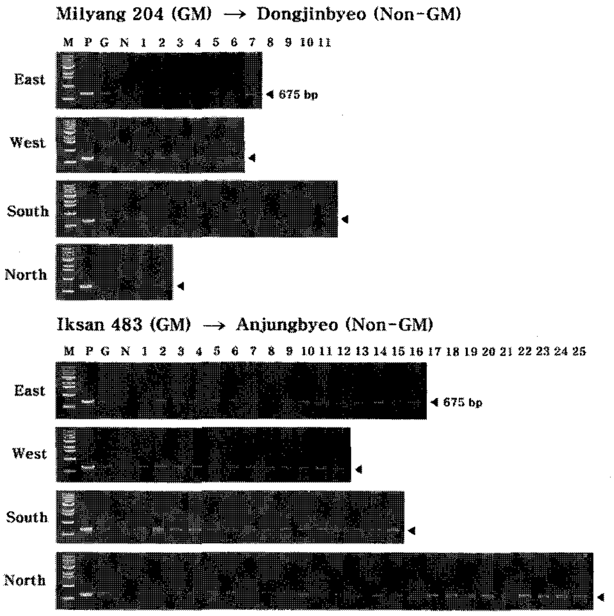

Figure. 2. PCR detection of the *bar* gene introgressed into non-GM rice from GM rice. P, Plasmid vector containing the *bar* gene; G, genetically modified variety; N, non-GM variety; Lane 1~25, F_1 hybrids through pollen-mediated gene flow from GM to non-GM rice.

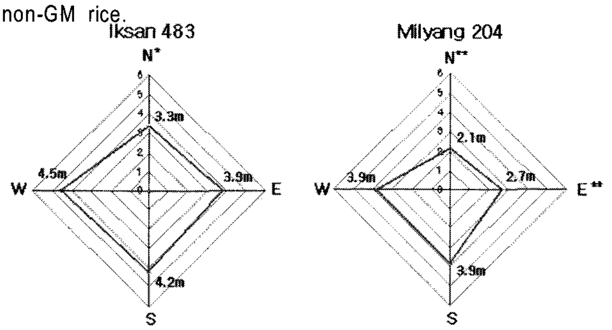

Figure. 1. Distance of pollen dispersal from GM rice (Iiksan 483 and Milyang 204) to non-GM rice (their wild types, Anjungbyeo and Dongjinbyeo). N, north; E, east; S, south; W, west. *,**Significant at P<0.01 and 0.05 to the maximum distance, respectively.

pollen grains was rapidly decreased below 50% at 5 minutes after shedding, and hardly gone out below 2.4% at 15 minutes after shedding. The viability of shed pollen grains in GM rice, Milyang 204 were not different to the female-parents, Junambyeo, but it showed a significant difference between Iksan 483 and its female-parents, Sindongjinbyeo at 10 and 15 minutes after shedding. Although the pollen viability of Iksan 483 was higher than the female-parents, it was slightly lower compared to Milyang 204 and Junambyeo. The viability of shed pollen grains in four GM and non-GM varieties surely lost at 30 minutes after shedding.

Pollen Ispersal and Outcrossing Rate from GM to Non-GM Rice

The levels of pollen-mediated gene flow to the glufosinate ammonium-tolerant GM rice (Iksan 483 and Milyang 204) were investigated by square-field trial designs using their wild types (Anjungbyeo and Dongjinbyeo) as the pollen recipients (Figure. 1, Table 3). When the recipient plants were placed to 6 m distance in the four directions of the edge of GM rice (3×3 m of square nucleus), the far distances of pollen dispersal in Iksan 483 were 3.9, 4.5, 4.2 and 3.3 m in east, west, south and north, and those in Milyang 204 were 2.7, 3.9, 3.9 and 2.1 m, respectively. The distance of pollen dispersal was significantly short in north direction both GM varieties. The direction of the dominant wind at flowering time was west-south, and the far distribution of the pollen-mediated gene flow was west and south both GM varieties (Iksan 483 and Milyang 204).

When the outcross of recipient plants was investigated in 1.5 m intervals from the edge of GM rice to 6.0 m (Table 3), the total numbers of GM hybrids were 67 and 26 plants in Iksan 483 and Milyang 204. The survival GM hybrids were detected the introgression of *bar* gene by electrophoresis of PCR products (Figure. 2). The outcross of two

Table 3. Outcrossing rate according to the distance of pollen disposal from GM rice (pollen donors, Iksan 483 and Milyang 204) to non-GM rice (pollen recipients, Anjungbyeo and Dongjinbyeo).

GM varieties	Direction	Total no. of GM hybrid and mean outcrossing rate (‰) by distance				
		≤1.5	~3.0	~4.5	~6.0 m	Total
Iksan 483	East	13(0.092)a^z	2(0.014)b	1(0.007)b	0(0.000)b	16(0.028)
	West	9(0.062)a	1(0.007)ab	2(0.014)ab	0(0.000)b	12(0.021)
	South	9(0.059)a	2(0.013)ab	4(0.027)ab	0(0.000)b	15(0.025)
	North	23(0.151)a	1(0.007)b	1(0.006)b	0(0.000)b	25(0.041)
	Total	53(0.091)a	6(0.010)b	8(0.014)b	0(0.000)b	67(0.029)
Milyang 204	East	6(0.047)a	1(0.005)b	0(0.000)b	0(0.000)b	7(0.013)
	West	3(0.022)a	2(0.014)ab	1(0.009)ab	0(0.000)b	6(0.011)
	South	6(0.047)a	2(0.015)ab	3(0.021)ab	0(0.000)b	11(0.021)
	North	0(0.000)b	2(0.013)a	0(0.000)b	0(0.000)b	2(0.003)
	Total	15(0.029)a	7(0.012)ab	4(0.007)ab	0(0.000)b	26(0.012)

LSD to outcrossing rate : Variety, 0.013** ; Direction, 0.018NS ; Distance, 0.018**

NS, **Non-significant or significant at P=0.01, respectively.

zMeans followed by same letter within a row are not significantly different at the 1% level, determined by LSD to outcrossing rate.

GM varieties showed a significant difference in varieties and distance, and the mean outcrossing rate was very low less than 0.003% (three plants per 100,000 grains). The outcrossing rates of Iksan 483 and Milyang 204 ranged from 0.021 to 0.041‰ and 0.003 to 0.021‰ in four directions at distance within 6.0 m, respectively. The outcrossing rate of Iksan 483 according to distance was significantly high within 1.5 m. The GM hybrids by the pollen dispersal did not detected over 4.5 m from the edge of GM rice.

Discussion

Growth characteristics of GM rice are not generally different to those of non-GM rice. There were not reports which the growth of GM rice was especially superior compared to the non-GM variety up to recently. In this study, Milyang 204 and Iksan 483 were not significant differences in growth characteristics compared to their non-GM female parents except heading date and 1,000 grain weight in Iksan 483. Two GM rice, Milyang 204 and Iksan 483 were not inferior in culm growth, panicle length, tiller number, filled gain rate and 1,000 grain weight compared to non-GM rice, Dongjinbyeo and Shindongjinbyeo. It seems that the difference of agronomic characteristics between GM and non-GM rice varieties are the genetic traits of rice varieties without regard to the introgression of *bar* gene.

The pollen-mediated gene flow from cultivated plants to their wild species has been recognized for a long time (De Candolle 1986), and Anderson (1949) discribed as the "super weeds" which meaned the noxious plants originated from the hybridization between cultivated plants and related wild species. The pollen-mediated gene flow had already well-known in various GM crops (Meier-Bethke and Schiemann 2003, Thompson et al. 1999, Arias and Rieseberg 1994). Patterns of pollen-mediated gene flow are vary according to biological factors such as pollination modes, pollen longevity and flowering season (McCartney and Lacey 1990, Westman et al. 2002), climatic factors such as wind speed and direction, atmosphere temperature and humidity (Khan et al. 1973, Giddings et al. 1997ab), and cultivated factors such as field position, population size and distance between the pollen donor and the recipient (Crawford et al. 1999, Thompson et al. 1999).

Pollen grains are a vector induced to gene flow from GM rice to non-GM cultivated, weedy and wild rice. Especially, the viability and longevity of pollen grains is one of the critical factors related to the production of GM hybrids between GM rice and cultivated, weedy or wild rice. In this study, the *in vitro* germination of fresh pollen in greenhouse (under 32±2℃ emperature, 65±3% humidity and 16,280± 1,268 lux of sunlight) was more than 90%, but it was largely decreased less than 2.4% in 15 minutes after shedding. The results showed that the pollen longevity of GM rice was rapidly decreased with the passing of time after shedding as well as non-GM rice varieties, and the longevity of shed pollen grains in four GM and non-GM varieties certainly lost at 30 minutes. We thought that pollen viability and longevity in cultivated rice was within 30 minutes after shedding as a varietal trait without regard to the introgression of *bar* gene. Song et al. (2003) also gained a similar result from cultivated rice, Minghui-63, and pollen longevity by the time to 0% germination was 30 minutes. However, pollen viability and longevity can vary greatly between species, and it is also dependent on temperature and humidity (McCartney and Lacey 1990). In wild rice and other grasses, the pollen viability and longevity was higher compared to the cultivated rice. The viability of fresh pollen in *O. rufipogon* was 60%, and the pollen longevity was 70 minutes (Song et al. 2003). In tall fescue, pollen viability reduced to 5% in 30 minutes, and it was completely lost in 90 minutes (Wang et al. 2004). The longevity of pollen grains based on seed set was also 5 hours after shedding in sorghums (Stephens and Quinby 1934) and sudan grass (Hogg and Ahlgren 1943). Maize pollen lost viability after 2 hours under field conditions (Luna et al. 2001). Thus, most of grasses with cross-pollinated system have a long pollen-longevity, while rice with self-pollination system has a short pollen-longevity by thin pollen wall (Fu et al. 2001). Above all, the rice plant with short pollen-longevity, short-term anthesis and self-pollination system may be beneficial to diminish the pollen dispersal from GM to non-GM rice field.

On the other hand, to assess the pollen-mediated gene flow, we detected the distance of pollen dispersal and outcrossing rate between GM and cultivated non-GM varieties. The maximum distances of out-crossed GM hybrids were 4.5 and 3.9 m from the edge of GM rice, Iksan 483 and Milyang 204 in west, and the shortest distances were 3.3 and 2.1 m in the opposite north. The maximum distance of gene flow is accord with the direction of the dominant wind at flowering time, and distance of pollen disposal and outcrossing rate are largely affected to the climatic and varietal differences such as wind direction and speed, atmosphere temperature and humidity, culm length, pollen longevity and flowering time between the pollen donor and the recipient plant (Chen et al. 2004, Messeguer et al. 2001, Song et al. 2003, Zang et al. 2003). In this study, the maximum distance of pollen disposal in Milyang 204 was shorter than that in Iksan 483, and the reason was due to the differences in flowering time (2 days) and culm length (21.1 cm) between the pollen donor and the recipient plant. The outcrossing rate between Iksan 483 and Milyang 204 showed a significant difference in distances and varieties. Outcrossing rate was greatest within 1.5 m from the edge of the GM rice field, and up to maximum of 0.015% outcrossing in north of Iksan 483. In rice with self-pollination system, the level of pollen-mediated gene flow can be largely affected by the degree of synchrony in flowering time and culm length between the pollen donor and the recipient plant. In this study, the outcrossing rate between Iksan 483 and Anjungbyeo which had a synchronized flowering time and a similar culm length was significantly higher than that between Milyang 204 and Dongjinbyeo with the different flowering time and culm length. The result showed that the synchrony of flowering time to assess pollen-mediated gene flow is one of the most important factors. And also, it is more desirable that the culm length of pollen donor varieties is similar or tall compared to pollen recipient in self-pollinated rice.

Generally, the cultivated rice with self-pollination system is known a low outcrossing rate less than 1% (Zhang et al. 2003), and the degree of outcrossing was generally higher in indica varieties and wild species than in japonica varieties (Oka 1988). In our study, the distance of gene flow was short and outcrossing rate was very low, and Song et al. (2003), Messeguer et al. (2001) and Zang et al. (2003) also detected low outcrossing rates less than 0.1% from GM to non-GM rice. While Lord (1935) reported high outcrossing rate, ranging 0.34 to 0.67% between cultivated rice varieties. In addition, the pollen-mediated gene flow between cultivated rice and weedy or wild rice was highly ranging 1 to 54% in side-by-side field conditions (Chen et al. 2004, Langevin et al. 1990, Oard et al. 2000, Song et al. 2003). Fortunately, wild rice was not over-wintering ecotype in Korea, but weedy rice (red rice, *O. sativa* f. *spontanea*) widely distributed from 0.6 to 23% in farmers' field, and it is a sevier problem in direct-seeded rice field because of reduction of yield and quality (Suh et al. 1997).

The herbicide-tolerant GM rice can be provide an efficient tool in weedy rice control, the sevier problem in direct-seeded rice field, but it involves a the risk to be introgressed the transgene to weedy rice. The progeny of GM hybrids can be a terrible weed, and the herbicide-resistant weedy rice is also the cause of seed contamination, yield reduction and quality deterioration as well as the contamination of herbicide-resistant GM seeds to non-GM conventional rice fields and organic rice fields (Noldine 1998, Pantone and Baker 1991). Moreover, the frequency of gene flow from GM rice to weedy and wild rice in larger populations can be much more increased than the result detected from our small size populations (3×3 m). Thus the gene flow from herbicide-resistant GM rice to weedy and wild rice species is one of serious risks in cultivation of GM rice. Accordingly, we thought that the effective strategies to prevent the pollen-mediated gene flow are the isolation of distance and the avoidance of flowering time between GM and non-GM rice, and the isolation distance have to consider wind direction and speed, field size and amount of pollen disposal.

Up to recently, various GM crops have been developed, and conducted field trials for commercial cultivation. GM rice among them can be beneficial to diminish the pollen dispersal of GM rice which has the short pollen-longevity and low outcrossing rate in our results compared to other crops (Arias and Rieseberg 1994, Giddings et al. 1997ab, Thompson et al. 1999, Luna et al. 2001, Westman et al. 2002). GM rice plants are able to spread vegetatively in tropical and subtropical areas, but rice ratoons are no risk of vegetative propagation because perishing of cold in winter season in Korea. In our study, the glufosinate ammonium-tolerant GM rice showed the similar risk in pollen longevity, distance of pollen disposal and outcrossing rate compared to the non-GM rice. Thus, the risk assessment of seed or pollen-mediated gene flow has to conduct the basic studies and field trials in detail, including such as biological, climatic and cultivated factors and the fitness of hybrids and progeny between GM and non-GM rice. Our results may help to reduce the risk of pollen-mediated gene flow in GM rice.

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