A system model for estimating regional cross-pollination rates between GM and non-GM rice considering actual meteorological conditions and field distributions

Seiichiro YONEMURA^{*,†}, Hiroyuki SHIBAIKE^{*}, Nobusuke IWASAKI^{*},
Shigeto KAWASHIMA^{**}, Tomoki USHIYAMA^{*††}, and Mingyuan DU^{*}

(^{*}National Institute for Agro-Environmental Sciences, 3–1–3 Kannondai, Tsukuba, Ibaraki, 305–8604, Japan)
(^{**}Kyoto University, Oiwakecho, Kitashirakawa, Sakyo-ku, Kyoto, 606–8502, Japan)

Abstract

To sustain a stable food supply without losses from various hazards, such as insects, diseases, or abnormal climate conditions induced by global change, application of genetically modified (GM) organisms is one potential tool for agriculture. However, GM crops do not exist in traditional agricultural environments; thus, the risks resulting from the application of GM crops to agriculture must be made clear. Crops, such as rice belonging to the Gramineae family, can be cross-pollinated by wind. This enables non-GM crops to be pollinated by GM crops. Prior to the cultivation of GM crops, the degree of cross-pollination must be estimated. This should take into consideration meteorological conditions, because of their importance in pollen flow. We constructed a system model to calculate the cross-pollination distribution by using data on the geographical distribution of GM donor and non-GM recipient crop fields, meteorological elements, and flowering data. The system consists of a main program to calculate cross-pollination rates, a program to include the isolation distance, and a sub-program to average the maps of the cross-pollination rates in recipient fields. The system can predict the regional cross-pollination rate by incorporating the isolation distance and differences in the flowering period. The system was applied to three areas around Tsukuba City, Ibaraki Prefecture, with different distributions of paddy fields and hypothetical donor (ratio 30%) and recipient (ratio 70%) fields. The general trend was that the cross-pollination rates were lower in areas of clustered donor fields. The calculated cross-pollination rate can mostly be explained by the length of the border between the donor and recipient fields. This is because rice pollen is dispersed only within short distances from donor fields. In order to clarify the influence of meteorological conditions on the variation in the cross-pollination rate, 10 years of simulations were performed. The cross-pollination rate varied by about a factor of three (0.03–0.09% for one of the simulated fields) during the simulated 10 years.

Key words: Genetically modified (GM) crop, Geographical Information System (GIS), Pollen Donor, Pollen Recipient, Regional-scale model.

1. Introduction

Genetically modified (GM) crops are a useful tool to help sustain a stable food supply by protecting it from various hazards, such as harmful insects,

diseases, or abnormal climate conditions induced by global change. The total area in which GM crops are planted is increasing rapidly all over the world. The USA, China, Australia, and some EU countries, such as Spain and Portugal, have introduced GM crops into their agriculture, whereas other countries such as Italy and Japan are refusing or hesitating to introduce GM crops. In particular, the USA is a leading nation in the development of GM crops and is eager to commercialize them worldwide. However, GM crops are

Received; December 15, 2010.

Accepted; March 14, 2011.

[†]Corresponding Author: yone@affrc.go.jp

^{††}Present address: Public Work Research Institute, 1–6 Minamihara, Tsukuba, Ibaraki, 305–8516, Japan

artificially produced and are not present under natural conditions, so the risks resulting from the application of GM crops to agriculture must be investigated. The risks are variable, reflecting the variability of the ecosystems in which GM crops might be grown. A study by Losey *et al.* (1999) raised concerns about the potential danger of introducing GM crops into ecosystems, suggesting that transgenes, such as those for insect resistance, might kill butterflies in areas surrounding the GM crops. Studies about the diffusion of artificial genes into non-GM crops or wild relatives are also important (e.g., Lavigne *et al.*, 1998, and subsequent papers).

Gene flow from GM to non-GM crops or wild relatives has been investigated in major crops, such as rapeseed, maize, rice, potato, sugar beet, and cotton (e.g., Messéan *et al.*, 2006). Each crop has different pollination processes, including self- and cross-pollination, and pollination by insects. The pollination of crops belonging to the Gramineae family such as wheat, maize, and rice is different from crop to crop: rice and wheat are more shifted toward self-pollination, whereas maize is more shifted toward wind-mediated pollination. However, it is important to study wind-mediated cross-pollination even for rice and wheat because they are major crops worldwide. Then, non-GM crops around GM crops can be pollinated and the degree of cross-pollination must be estimated before the usage of GM crops.

Pollen flow from GM maize has also been well studied (Jarosz *et al.*, 2003; Jarosz *et al.*, 2005; Kawashima *et al.*, 2004; Sanvido *et al.*, 2008; Ushiyama *et al.*, 2009), because *Bt* (*Bacillus thuringiensis*) maize has high productivity due to the reduction of insect damage and because cross-pollination can occur over long distances (hundreds of meters or more). Some of the above studies in Europe were conducted in projects that aim to promote the introduction of GM crops. One of these was SIGMEA (Sustainable Introduction of GM Crops into European Agriculture) (EU Project no. 501986), a cross-disciplinary European Policy-Oriented Research Project that started in May 2004 and finished at the end of 2007 (<http://www.inra.fr/sigmea>).

The EU (Messéan *et al.*, 2006) produced a summary report, synthesizing previous studies and summarizing measures to be taken to reduce cross-pollination between GM and non-GM crops under standard agricultural practices. The EU has empirically determined the maximum allowable rate of the adventitious

presence of GM seed to be 0.9%, whether from pollination (hybridization in fields) or mixture during the harvesting process. Although the standpoint of each country is different, reflecting the culture of its citizens, the report is being used as the basis for policy development by EU countries.

To estimate gene flow via pollen from GM to non-GM maize, Messéan *et al.* (2006) employed a model tool (MAPOD; Matricial Approach to Pollen Dispersal) that was later reported in detail (Angevin *et al.* 2008). In MAPOD, the flowering dynamics and pollen dispersion between GM and non-GM maize crops were simulated and influencing factors such as the size and shape of the fields, distribution of GM and non-GM fields in the agricultural landscape, and flowering dates and dynamics are integrated. Model parameter values of MAPOD were either derived from existing models of pollen dispersal or estimated from experimental field studies. After the authors estimated regional cross-pollination rates for maize, they suggested measures to be taken to reduce the overall amount of cross-pollination. The first important measure is to have an adequate isolation distance between GM and non-GM maize; the second is to have a difference in the flowering period. The authors produced a decision table for determining the isolation distances necessary to keep the adventitious GM presence rates due to cross-pollination below a defined threshold for a certain area of GM maize; the table included downwind and upwind cases.

For rice, there have been some studies in Southern Europe (e.g., Messeguer *et al.*, 2001). In Japan, cross-pollination between glutinous and non-glutinous rice varieties has been investigated by measuring xenia (cross-pollination visible in the endosperm) in connection with grain quality (Nishiyama *et al.*, 1973; Saito and Iyama, 1973; Ito, 1986; Kamagata *et al.*, 1990). Unfortunately, most of these articles are only written in Japanese and therefore cannot be easily referred to by foreign researchers. Kumar *et al.* (2008) summarized information about Indian rice and suggested that gene transfer from herbicide-resistant rice is considered to be especially high in India, where cultivated rice and its relatives are sympatrically distributed and their flowering times overlap. The most intensive field studies have been conducted in China, which is very eager for the commercial production of GM rice (Jia *et al.*, 2004; Lei, 2004; Lu and Snow, 2005). Experimental and field studies of cross-pollination rates

under various conditions have been intensively and extensively conducted (Song *et al.*, 2003; Chen *et al.*, 2004; Rong *et al.*, 2004; Rong *et al.*, 2007). Rong *et al.* (2010) produced an empirical model that showed an exponential increase in the amount of pollen received by recipient plants with a decreasing distance from donor plants.

In 2001, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan began projects to assess the impact of GM crops on the environment and ecosystems and to consider the coexistence of GM and non-GM crops. At the beginning stage, field studies were intensively conducted for many crops, especially maize (Kawashima *et al.*, 2004). Later, Ushiyama *et al.* (2009) used a Lagrangian model to calculate maize pollen dispersion more dynamically by using experimental data. Moreover, based on results from field experiments, Kawashima (2006) developed a prototype model to calculate rice pollen dispersion (see Section 2 of this paper). The most striking difference between the models (Kawashima, 2006; Rong *et al.*, 2010) is that Kawashima (2006) has a physical basis of plume model, whereas Rong *et al.* (2010) used empirical exponential fittings for their model.

Before considering actual large-scale application of GM rice in paddy fields, it is important to regionally estimate cross-pollination using a system model that incorporates actual field conditions, even though it is very difficult to estimate regional cross-pollination rates by field experiments. Actual questions include how regional-scale cross-pollination rates depend on various environmental conditions; what kind of fields or donor and recipient distribution (land use) can minimize regional cross-pollination rates; and how the isolation distance can lower regional cross-pollination rates. To answer these questions, we applied our model (Kawashima, 2006) to simulate cross-pollination rates in fields constructed from geographical data. We prepared data sets for three areas as examples of the different kinds of land uses.

2. Structure of the system model

The system consists of three parts: the main program *pdhm2*, the isolation-distance-setting program *kakuri*, which means “isolation”, and the regional-averaging program *summarize*. We used the three programs to systematically calculate the average cross-pollination rate (CPR) under various conditions.

2.1 Main program

The main program, *pdhm2*, is the core model of the system. The core model was made to calculate pollen dispersal and cross-pollination in rice (Kawashima, 2006) (Fig. 1). The original code of *pdhm2* was written in the FORTRAN programming language but was converted to the C language for more flexibility in programming. The *pdhm2* program reads map data with donor and recipient locations, 10 days of meteorological data, and 10 days of flowering data, and calculates the CPR at each cell of the grid. *pdhm2* uses a plume model, which is a kind of quasi-mechanistic model that has been widely used for the prediction of diffusion of atmospheric pollutants. The model has the advantage of rapid calculation time, in contrast to the Lagrangian model (Ushiyama *et al.*, 2009), which traces the movement of each particle one by one. *pdhm2* has flexibility with respect to grid size and calculation time from the beginning to the end of the flowering that is set by the header file *pdhm.h*. Wind direction is obtained from meteorological data. For the flexible design of field distribution of donors and recipients, the *pdhm2* program can easily incorporate changes of wind direction (in degrees) throughout the simulation period. In this study, we set the grid resolution as 10 m.

2.2 Averaging program and isolation-distance-setting program

The program *summarize* obtains a regional CPR by averaging the CPRs across the grids in the recipient fields obtained by *pdhm2*. The *summarize* program obtains the grid information from the recipient field's

Fig. 1. Schematic diagram to calculate CPR at each time point (one-dimensional image). The grey section under the curve is used for the calculation of CPR averages.

distribution file and averages the CPRs in the recipient fields.

To calculate the regional CPR over an area excluding that within a particular isolation distance from the donor fields, the area of the recipient fields outside the isolation distance from the donor field is selected and used to create a new recipient field map. This is done with the program *kakuri*, which masks the recipient fields within a given isolation distance from the donor fields.

2.3 Data incorporation into the system model

Two types of data from the geographical information system (GIS) were intensively prepared from various sources for the input data of the system: fine-scale data (“fine data”) and standard-scale data (“std data”). The GIS data were produced with GRASS (GRASS Developer Team, 2010 <http://grass.fbk.eu/>) and Quantum GIS (Quantum GIS Developer Team, <http://www.qgis.org/>).

The fine data were derived from 1:2500-scale national basemaps published by each city. The land-use boundaries and road edges on the maps were digitized, and land-use categories were assigned to each polygon. The good spatial and classification accuracy means that the fine data are suitable for the assessment of regional cross-pollination. However, it requires a lot of time and effort to produce fine data. Thus, there is a need to produce GIS data in a more efficient way.

The std data were produced by combining GIS data about agricultural land use and geographical features such as roads, canals, rivers, and railways. The data about agricultural land use were derived from “The 4th Fundamental Research of Land Use Infrastructures” (FRLI) which was provided by MAFF. The data on geographical features were derived from “Fundamental Geospatial Data” (FGD) provided by the Geospatial Information Authority of Japan. The FRLI data didn’t include enough information on features such as roads and so to address this, the FGD data were superimposed on the FRLI data and used to divide the agricultural land into parcels. The spatial coverage of FRLI and FGD is the whole area of Japan. Therefore, the std data are easily produced for any area of Japan. However, the spatial accuracy of both input data sets is coarse: FRLI is 1:50 000 and FGD is 1:25 000.

The fine and std maps of the three investigation areas, Area 1, Area 2, and Area 3, around Tsukuba City, Ibaraki Prefecture, were prepared (Fig. 2). The size of each area is 3 km×4 km, containing 300×400

Fig. 2. Distribution of donor (white) and recipient (gray) fields. Left column, fine data; right column, std data. Black indicates land not used for paddy field. Each area is 4 km east–west and 3 km north–south.

10-m-square cells. Area 1 is situated in the suburb of Tsukubamirai City and contains part of the Kokai River. Area 1 has large agricultural paddy fields. Area 2 is situated in the northwestern suburbs of Tsukuba City. Area 3 is situated in the northeastern suburbs of Tsukuba City at the base of Tsukuba Mountain. Area 3 has many smaller paddy fields. For our model, 30% of the paddy field area was randomly allocated as donor fields (*i.e.*, simulated GM-fields); the remaining 70% of the paddy fields were assigned as recipient fields (*i.e.*, simulated non-GM fields).

The *pdhm2* model was validated for the intensive field data with full data sets of meteorology, pollen numbers by a Durham Sampler, optically-measured pollen counts, and pollination data. Cross-pollination was assessed by xenia observed in the endosperm and PCR gene data that were collected by our institute in 2005. The NIAES 2005 intensive data were used as the default data. A total of 10 years of the same meteorological data set at Tsukuba was taken from the Tateno meteorological station at Tsukuba with the linear-transfer correction of wind speed. This is because wind speed is critically important in the diffusion of pollen.

From our previous field experiments, two pairs of the following variation-to-variation flowering data that could be applicable to our model were parameterized.

Glutinous and non-glutinous varieties were used in our previous experiments to quasi-simulate cross-pollination by GM rice. The pair of varieties with lower CPR consisted of *Okunomurasaki* and *Himenomochi*, and the pair of varieties with higher CPR consisted of *Nipponbare* and *Heiseimochi*. We applied the higher parameter to our model through the flowering data.

3. Examples of application of the system model

The averaged CPR in maps with a smaller field size (area 3) was higher than in those with larger fields (area 1, area 2) (Table 1); the averaged CPR of the fine maps was three times that of the std maps (Fig. 3, Table 1). The meaning of these two findings is essentially the same: maps with clusters of the same type of field showed smaller CPR. The strategy of clustering GM fields should be taken when GM rice is grown in actual paddy fields. It should be noted that the fine data are recommended to be used for CPR calculations for rice because of its short-distance cross-pollination, although it appears that the calculated CPR using std data is lower. Some corrections are necessary to estimate regional CPRs when using the std data.

To consider the cross-pollination between donor and recipient fields, local meteorology, especially wind speed and direction, is very important. There are some regions where the wind direction has special characteristics; therefore, calculations performed with changes in the wind direction are useful in regions

where sea-to-land winds or mountain-valley winds periodically change direction. For this reason, we made it possible to change wind direction by a certain angle (“angle of deviation”) in our model. We show how CPR depends on the angle of deviation from the original wind direction in Fig. 4. The CPRs of both the fine and the std data showed rectangular shapes with peaks at around 150° and 320° angles of deviation. The rectangular shapes of the CPR distribution are considered to reflect the clearly-distributed rectangular large fields in area 1.

Our application of 10 years of meteorological data at the Tateno meteorological station at Tsukuba with linear correction of wind scale showed similar variation for both the fine and std data, *e.g.*, about a factor of three (0.03–0.09%) for the fine data in area 1 (Fig. 5). The direct average of the meteorological wind speed data during the flowering period does not seem to be directly related to the CPR. This is possibly because flowering is a day-by-day phenomenon, so a simple average of wind speed over the season is not a good parameter for predicting cross-pollination. Understanding the degree of variation is necessary to evaluate the confidence interval of the estimated CPR values because meteorology cannot be predicted precisely and precisely because, in practice, this means that simulations must be conducted for various likely scenarios. Application of long-range meteorological data at different places in the system is important for

Fig. 3. Distribution of calculated CPR for fine (left) and std (right) data for area 1. Black indicates land not used for paddy fields; white indicates donor (simulated GM) rice fields. Other colors represent recipient (simulated non-GM) rice fields: purple represents little cross-pollination, and remaining colors are shown in the bar below each figure. Recipient fields have high CPR only in areas adjacent to donor fields, reflecting the short-distance pollination of rice.

Table 1. Averaged CPR (%) for each map data

area	fine	std
area1	0.0182	0.0052
area2	0.0167	0.0062
area3	0.0207	0.0081

the actual assessment for the application of GM crops in future studies.

The use of our system model required a more powerful computer than a typical desktop computer. However, if a simple index showed a good correlation with the CPR calculated by the system model, it would be convenient for the evaluation of the CPR in the field. Cultivated rice is basically autogamous (self-pollinated) and the pollination distance is limited to short distances (e.g., 0.1% cross-pollination at 1 m) from the donor fields (Ito, 1986; Rong *et al.*, 2007). So when the donor and recipient fields are randomly distributed within a large map, it can be expected that the length of the border between donor and recipient fields would be an important parameter for calculating the average CPR (*i.e.*, the CPR would be directly proportional to the width of the area of contact between fields). We therefore calculated these total lengths and average lengths for the areas in this study. Two methods of calculation of the average lengths can be considered—one is to first calculate the average length of each field, and then calculate the average of those values (AL1); the second is to calculate the total field length and total field area, and then calculate the average length (AL2). Based on the R^2 values obtained,

Fig. 4. Distribution of CPR for area 1 fine and std maps based on angle of deviation.

Fig. 5. Yearly time-series of calculated CPR for fine data sets. Calculated CPRs for std data are not shown because of their similarity to the fine-data CPRs.

Fig. 6. Relationship between field-length indices and averaged CPRs. Correlations in cases 1, 2, 3, and 4 are for AL1 std, AL2 std, AL1 fine, and AL2 fine, respectively.

AL1 seems to be a better index than AL2 (Fig. 6). Furthermore, as noted previously, the differences in the CPR between the fine and std maps reflect the degree of clustering, because of less "contact" between donor and recipient fields due to the larger field sizes in the std maps. When the fine and std data were combined, better correlations were obtained. Thus, these indices can be considered to be good candidates for the simplification of regional CPR estimation. The distribution of donor and recipient fields was randomly assigned in this study, but a combination with the properties of wind direction and land use topography may give even more satisfactory results. The indices described here can be applied to crops such as wheat that are also generally self-pollinated (autogamous), but would not be suitable for maize, which is more often cross-pollinated and is pollinated at longer distances.

4. Discussion and Concluding Remarks

This study developed a system model to systematically calculate regional rice CPRs; it extends the estimation of CPRs of rice in recipient fields from field-scale data (Kawashima, 2006) to a regional scale by incorporating GIS map data. To our knowledge, this is the first study in the world to estimate regional CPRs for rice. The original model (Kawashima, 2006) in this study has a physical basis and therefore our system is believed to be robust. Furthermore, the parameters of this system model such as grid size, meteorological data, and map data can be easily changed, and our system model is very flexible, so it can be applied to different kinds of field conditions.

We used two types of data sets: std and fine. It should be noted that fine data are recommended to be used in CPR calculations for rice because of its short-distance pollination (Table 1), except in the case of highly clustered fields. This difference between the fine and std data is more pertinent for rice than for other crops, such as maize, that have long-distance pollination.

The EU (Messéan *et al.*, 2006) has estimated the regional variation of CPRs for maize. It appears that their maps of CPRs are based on the field level and are not gridded within fields. In our case, a fine-gridded calculation was necessary because the cross-pollination of rice occurs within short distances.

This is the first study to calculate regional-scale CPR for rice, so the submodels will need further improvements. For example, it is likely that more parameters of cross-pollination between various rice varieties are necessary in our simulation. We assigned the distribution of donor and recipient fields in the maps at a constant ratio, so additional patterns of distribution could be themes for further studies. It should be noted that validation and improvement is still under construction even for the original model (Kawashima, 2006; Kawashima and Hama, 2010) and regional-scale validation remains a difficult challenge.

To predict the CPR before the seed set when GM rice cultivars are planted in actual fields, it will be necessary to first predict regional meteorology, and then to predict plant growth (*i.e.*, the flowering time) before the use of our system model. In the future, the coupling of our system model and a crop growth model with predicted meteorology will provide a good tool for practical use. Furthermore, more updating of

our model will be necessary to enable decision-making in administrative sections prior to the introduction of GM rice into an area.

Acknowledgement

This study was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Assurance of Safe Use of Genetically Modified Organisms Project).

References

- Angevin, F., Klein, E. K., Choimet, C., Gauffreteau, A., Lavigne, C., Messéan, A., and Meynard, J. M., 2008: Modelling impacts of cropping systems and climate on maize cross-pollination in agricultural landscapes: The MAPOD model. *Eur. J. Agron.*, **28**, 471–484.
- Chen, L. J., Lee, D. S., Song, Z. P., Suh, H. S., and Lu, B. -R., 2004: Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. *Ann. Bot.*, **93**, 67–73.
- Ito, T., 1986: On the natural crossing of rice plant. *Bulletin of the Mie Agricultural Research Center*, **14**, 1–6 (in Japanese).
- Jarosz, N., Loubet, B., Durand, B., McCartney, A., Foueillassar, X., and Huber, L., 2003: Field measurements of airborne concentration and deposition rate of maize pollen. *Agric. For. Meteorol.*, **19**, 37–51.
- Jarosz, N., Loubet, B., Foueillassar, X., and Huber, L., 2005: Variations in maize pollen emission and deposition in relation to microclimate. *Environ. Sci. Technol.*, **39**, 4377–4384.
- Jia, H., Jayaraman, K. S., and Louet, S., 2004: China ramps up efforts to commercialize GM rice. *Nat. Biotechnol.*, **22**, 642.
- Kamagata, T., Hatakeyama, T., and Fujishiro, J., 1990: Studies on the reversion of glutinous seeds to non-glutinous seeds in seed production culture of paddy rice. II Varieties and preventive measures against natural outcrossing. *Bull. Chiba Found. Seed and St. Farm*, **12**, 1–7 (in Japanese).
- Kawashima, S., 2006: Modelling and simulation of pollen dispersal and hybridization processes. Proceedings of Workshop 4: Ecological Risk Assessment for the Gene Flow from the Genetically Modified Crops, NIAES International Symposium 2006, pp. 190–200.
- Kawashima, S., Matsuo, K., Du, M., Takahashi, Y., Inoue, S., and Yonemura, S., 2004: An algorithm

- for estimating potential deposition of corn pollen for environmental assessment. *Environ. Biosafety Res.*, **3**, 197–207.
- Kawashima, S., and Hama, T., 2010: Pollen dispersal and hybridization model for risk assessment of genetically modified crops. *Proceedings of the International Technical Meeting on Air Pollution Modelling and its Application*, NATO, Torino, September 2010.
- Kumar, V., Bellinder, R. R., Brainard, D. C., Malik, R. K., and Gupta, R. K., 2008: Risks of herbicide-resistant rice in India: A review. *Crop Protection*, **27**, 320–329.
- Lavigne, C., Klein, E. K., Vallee, P., Pierre, J., Godellée, B., and Renard, M., 1998: A pollen-dispersal experiment with transgenic oilseed rape. Estimation of the average pollen dispersal of an individual plant within a field. *Theor. Appl. Genet.*, **86**, 886–896.
- Lei, X., 2004: China could be first nation to approve sale of GM rice. *Science*, **306**, 1458–1459.
- Losey, J. E., Rayor, L. S., and Carter, M. E., 1999: Transgenic pollen harms monarch larvae. *Nature*, **399**, 214.
- Lu, B. -R., and Snow, A. A., 2005: Gene flow from genetically modified rice and its environmental consequences. *Bioscience*, **55**, 669–678.
- Messéan, A., Angevin, F., Gomez-Barbero, M., Menrad, K., and Rodriguez-Cerezo, E., 2006: New case studies on the coexistence of GM and non-GM crops in European agriculture, European Commission/Joint Research Centre, EUR22102EN, 112 pp.
- Messeguer, J., Fogher, C., Guiderdoni, E., Marfà, V., Català, M. M., Baldi, G., and Melé, E., 2001: Field assessments of gene flow from transgenic to cultivated rice (*Oryza sativa* L.) using a herbicide resistance gene as tracer marker. *Theor. Appl. Genet.*, **103**, 1151–1159.
- Nishiyama, H., Motomura, H., Okada, M., and Lee, S. K., 1973: On the contamination of nonglutinous grains in glutinous rice varieties caused by natural crossing. *Kyushu Agric. Res.*, **35**, 54–56 (in Japanese).
- Rong, J., Lu, B. -R., Song, Z., Su, J., Snow, A. A., Zhang, X., Sun, S., Chen, R., and Wang, F., 2007: Dramatic reduction of crop-to-crop gene flow within a short distance from transgenic rice fields. *New Phytol.*, **173**, 346–353.
- Rong, J., Song, Z., de Jong, T. J., Zhang, X., Sun, S., Xu, X., Xia, H., Liu, B., and Lu, B. -R., 2010: Modelling pollen-mediated gene flow in rice: risk assessment and management of transgene escape. *Plant Biotechnol. J.*, **8**, 452–464.
- Rong, J., Xia, H., Zhu, Y., Wang, Y., and Lu, B. -R., 2004: Asymmetric gene flow between traditional and hybrid rice varieties (*Oryza sativa*) indicated by nuclear simple sequence repeats and implications for germplasm conservation. *New Phytol.*, **163**, 439–445.
- Saito, S., and Iyama, J., 1973: Some investigations on the appearance of non-glutinous rice grains in glutinous rice plant varieties. *Kyushu Agric. Res.*, **35**, 57–58. (in Japanese).
- Sanvido, O., Widmer, F., Winzeler, M., Streit, B., Szerencsits, E., and Bigler, F., 2008: Definition and feasibility of isolation distances for transgenic maize cultivation. *Transgenic. Res.*, **17**, 317–335.
- Song, Z. P., Lu, B. -R., Zhu, Y. G., and Chen, J., 2003: Gene Flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. *New Phytol.*, **157**, 657–665.
- Ushiyama, T., Du, M., Inoue, S., Shibaike, H., Yonemura, S., Kawashima, S., and Amano, K., 2009: Three-dimensional simulation of maize pollen dispersal and cross-pollination, and the effects of windbreaks. *Environ. Biosafety Res.*, **8**, 183–202.