Modeling Pollen-Mediated Gene Flow in Rice: Implications for Assessing and Managing Transgene Escape

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Rice and rice gene pool

Asian cultivated rice (*Oryza sativa* L., referred to hereafter as rice) is an important world crop, providing staple foods for nearly one half of the global population. Achieving high productivity, including improved breeding methods, is always the challenge in meeting the demand for food security and nutritional sufficiency, particularly for the developing world. Apart from Asian rice, there is another cultivated rice species (*O. glaberrima*), domesticated in West Africa, that remains only locally important in some areas. In addition to the two cultivated species, there are more than 20 wild relative species in the genus *Oryza* and about 50 other wild species in the tribe *Oryzeae* of the grass family (Poaceae). All wild species in the tribe compose a valuable rice gene pool that is significant for providing germplasm for the genetic improvement of rice cultivars, in addition to other ecological functions. Species in the genus *Oryza* contain ten different genome types (i.e., AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH, and JJKK genomes), reflecting their relatedness to each other in terms of evolution. Rice contains the AA genome, which is shared by a group of six wild (including weedy) *Oryza* species that have the closest evolutionary relationships to the crop. Consequently, pollen-mediated gene flow or hybridization occurs naturally between rice and its close wild relatives to various extents.

Genetically engineered rice and its biosafety concerns

Demand for high-yielding and efficient rice production and rapid progress in transgenic biotechnology have prompted genetically engineered (GE) rice research and development. To date, many GE rice lines conferring unique traits have been developed in different countries, and some lines have been released into the environment for biosafety assessments. As the world’s largest rice producing and consuming country, China has actively engaged in GE rice research and development. Recently, two GE rice lines were approved by biosafety authorities in China for commercialization, and many more lines are in the pipeline. Like many other GE crops, the forthcoming extensive environmental releases and commercial production of GE rice have already aroused tremendous biosafety concerns worldwide. Among these concerns are transgene escape through pollen-mediated gene flow (PMGF) from GE rice varieties to non-GE rice and wild relatives and their potential (undesirable) environmental impacts. These concerns have become one of the most debated ecological biosafety issue, which has considerably constrained the commercialization and wide application of GE rice. Such biosafety concerns caused by transgene flow should be fully addressed with supporting and solid data, based on rigid scientific methodologies.

Pollen-mediated transgene flow and its potential environmental impacts

Previous studies have concluded that PMGF occurs frequently between rice varieties, as well as between rice and common wild rice (*O. rufipogon*) or weedy rice (*O. sativa f. spontanea*). The movement of transgenes from a GE rice variety to its non-GE counterpart can trigger disputes over trading and legal issues, due to the so-called “contamination” of non-GE rice varieties. Although common wild rice is the ancestor of rice and the most important germplasm for rice breeding, transgene escape from GE rice to wild relatives via PMGF can potentially increase the ecological fitness or invasiveness of wild populations, resulting in serious weed problems. Similar problems may also be caused by transgene flow to weedy rice that is already a noxious weed in rice fields. Conversely, such transgene flow may lead to the reduction or even extinction of local wild rice populations if the transgene reduces the fitness of wild populations. Rice is sexually compatible with all close wild rice species containing the AA genome, indicating that the likelihood of PMGF from GE rice to these wild relatives is relatively high. Given the importance of the frequency of crop-to-crop or crop-to-wild transgene flow that determines the magnitude of environmental impacts, accurate determination of PMGF will greatly facilitate the assessment and management of potential impacts caused by transgene escape in rice.

Modeling pollen-mediated transgene flow in rice

A considerable amount of data on PMGF in rice have been generated from field experiments worldwide, providing useful information for the basic understanding of transgene flow from GE rice to its non-GE counterparts and to wild/weedy
reduce crop-to-wild PMGF, because most common wild rice populations are relatively small and fragmented. mon wild rice occurs continuously with a high population density. Therefore, more effective isolation should be required to used to calculate the conspecific pollen density in the wild rice population, the model simulation actually assumes that com to examine if this affects our calculations or that the effect is too small to be of quantitative importance. When the integral is a fat-tailed model should fit better the data for long distances. Additional data for long-distance dispersal could be collected > 100 m from sources, pollen is still found and the amount is more or less consistent. If the amount of pollen is significant, The exponential function fitted the pollen flow data excellently within the experimental scale (about 100 m). At distances of level (< 1%) requires a long isolation distance because the outcrossing rate of common wild rice could be as high as 40%. A greater isolation distance is required for small recipient plots than large recipient plots. In production, therefore, only a short isolation distance (a few meters) is required in rice to achieve a significant reduction of PMGF to frequencies of < 0.9% if large recipient plots are deployed, minimizing the potential ‘contamination’ from GE rice. Simulations are made in order to predict PMGF more precisely under different climate conditions. Then, we established a quasi-mechanistic PMGF model using the empirical pollen dispersal pattern as a baseline, and adding the outcrossing rate of a recipient and cross-compatibility between rice and pollen recipients. In the model, a fixed fraction (s) of the ovules is self-pollinated prior to the arrival of alien pollen. The highest possible outcrossing rate t equals 1−s. The remaining ovules can be fertilized by alien or self pollen, depending on the ratio of the pollen that reaches the stigma. Actual outcrossing rates will be lower than the maximum t, and more so when self pollen is abundant.

The PMGF frequency via outcrossing also relies on cross-compatibility between the pollen donor and recipient. This model represents well previous PMGF data from rice and common wild rice; therefore, it provides a solid base for understanding the pattern of PMGF influenced by climatic and biological factors. If a model only uses empirical functions to describe relationships between frequencies of PMGF and spatial distances, it will have a relatively low predictive power, especially for crop-to-wild gene flow with evident reproductive barriers. Simulation results indicated that without consideration of such biological parameters, the model cannot avoid overestimation of PMGF from the crop to wild relatives. In addition, the PMGF model also addresses the concerns of the ‘size effect’ of using PMGF data obtained from controlled experiments and extrapolating to a large field production scale. Our model simulations showed that in a self-pollinating species, PMGF increased with the increase of pollen source size, but such a size effect leveled off quickly with increasing pollen source size. Such a “size effect” approached an upper limit when pollen source size became infinite. Thus, we can estimate PMGF on a large field scale using the PMGF model.

**Implications of PMGF model for assessing and managing transgene flow**

We explored the possibility of using the PMGF model as a practical tool for assessing and managing transgene escape in rice. The model can estimate the isolation distances required for minimizing the frequency of crop-to-crop and crop-to-wild PMGF. Under the worst case scenario of crop-to-crop gene flow, where the recipient plot is surrounded by pollen source plots with infinite sizes and pollen can easily disperse long distances, the size of a recipient plot has a meaningful impact: a greater isolation distance is required for small recipient plots than large recipient plots. In production, therefore, only a short isolation distance (a few meters) is required in rice to achieve a significant reduction of PMGF to frequencies of < 0.9% if large recipient plots are deployed, minimizing the potential ‘contamination’ from GE rice. Simulations are made under the assumption of extremely limited reproductive barriers between rice varieties. Given the observed reproductive barriers between some varieties, particularly between the two subspecies *indica* and *japonica* rice, the frequency of PMGF may still be overestimated, compared with the actual situation in rice fields.

Under the worst case scenario, the attempt to reduce the frequency of PMGF from rice to common wild rice to a low level (< 1%) requires a long isolation distance because the outcrossing rate of common wild rice could be as high as 40%. The exponential function fitted the pollen flow data excellently within the experimental scale (about 100 m). At distances of > 100 m from sources, pollen is still found and the amount is more or less consistent. If the amount of pollen is significant, a fat-tailed model should fit better the data for long distances. Additional data for long-distance dispersal could be collected to examine if this affects our calculations or that the effect is too small to be of quantitative importance. When the integral is used to calculate the conspecific pollen density in the wild rice population, the model simulation actually assumes that common wild rice occurs continuously with a high population density. Therefore, more effective isolation should be required to reduce crop-to-wild PMGF, because most common wild rice populations are relatively small and fragmented.
The established PMGF model can be used to predict transgene flow under diverse conditions, which provides a useful tool to determine isolation distances between GE rice and non-GE counterparts or wild relatives to minimize transgene flow. Given that pollen dispersal of other wind-pollinated crops (e.g., wheat and barley) is similar to rice, and the key biological parameters required by the model can be easily estimated from field experiments, the PMGF model has a good potential for use in these crops.

References

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