



Plant Gene Containment

Edited by Melvin J. Oliver and Yi Li

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Plant Gene Containment

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Foreword

World Food Supply

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The United Nations projects that by 2050 world agriculture will need to increase food production by 70% (The United Nations Food and Agriculture Organization, 2010) in order to feed a projected world population of approximately 9 billion. Even with the increase in food production, the world will still have more than 1 billion undernourished people and more than 100 million living close to starvation. Therefore, in reality, world food production needs to be increased by more than 70% in order to have any impact on decreasing world hunger. However, the many claim that there is no longer a major food problem as evidenced by improvements in a decrease in the percentage of poor people. However, the numbers tell a different story since there will still be more than 1 billion impoverished people when the world population reaches approximately 9 billion, even though the percentage of impoverished people will have decreased. In addition, food imports will continue to dramatically increase; for example, wheat imports are projected to increase from

30 to 75 million metric tonnes (MT) by 2020 (Pingali and Rosegrant, 1998). It is clear that extraordinary improvements in world food production will be necessary.

Domestication of all the major food crops revolutionized human culture by allowing for a decrease of hunter-gatherer societies and the development of villages, then towns, cities, and finally countries. Once domesticated, plants grown for food have been continually improved and selected for increased production. The improvements in world food production have been steady over the past 40 years, ranging from ~2.63 billion metric tonnes (BT) in 1963 to ~7.99 BT in 2005 (including plants 6.90 BT and animals 1.09 BT) (The United Nations Food and Agriculture Organization FAOSTAT, 2010). Most importantly, this massive increase in food production was accomplished basically on the same amount of land. The dramatic increase in plant food production mainly came from improved crop cultivars, crop technology advances, and better management practices (World Bank World Development Report, 2008).

To feed the world's increasing population, we will be required, first, to not only increase food (plant) production but also do it in a manner that will improve world dietary standards. Second, the world has to start addressing the overwhelming task of equitably distributing food to all regions of the world in order to offset increasing world hunger in developing countries. We will never see a lasting solution to the world hunger problem without a strong balance between food production and distribution—in other words, social justice. Third, we will need to accomplish these objectives with a minimum, or even better, a positive impact on the world's environment. Fourth, most importantly, we will need to continue increasing world food production without expanding the land currently under cultivation. The increase in food production between the

years 1963 and 2005 was accomplished without increasing the amount of arable land under production. For example, world grain yields more than doubled from 1.4 T/ha in 1961-1963 to 3.05 T/ha in 1997-1999, on approximately 56% less land (World Bank World Development Report, 2008). A 70% increase in world food production would equal approximately 23% of the current world production. Therefore, increases in world food production between now and 2040 are feasible by utilizing existing and newly developed technology to improve cultivar and management development without any further damage to our environment. However, we should keep firmly in mind that world food production increases could be subject to a number of additional undefined constraints.

This discussion suggests the potential for continued increases in world food production based on existing and newly developed technology; however, there are several limitations that could influence any increase in world food production. First, there might be limited access to technology for advancing yield to all regions of the world where food demands exist. Second, advanced technology and management inputs could easily spread into areas where environmental problems would be accelerated resulting in an adverse impact on the environment and biodiversity. Third, the public understanding of modern technology for increasing food production certainly needs to be improved. Major food production increases will certainly have to be based on the utilization of modern technology. To obtain the 7.99 BT of food produced in 2005 using 1963 cultivar/management technology (~2.63 BT) (The United Nations Food and Agriculture Organization FAOSTAT, 2010) would have required an additional ~1.6 billion hectare of new land brought under cultivation, which would have had a massive negative impact on existing biodiversity and our environment. Intensifying agricultural technology on

existing lands, therefore, will continue to play a major role in preserving biodiversity and maintaining the sustainability of our fragile global environment.

It is clear that, to eliminate hunger, we must increase sustainable world food production. One vital need involves our continued preservation of sufficient genetic diversity in plants and their relatives to ensure that the capacity to create cultivars capable of resisting new biotic and abiotic stresses and at the same time adapting to new environmental conditions is maintained. Existing and newly developed biotechnological tools alongside traditional plant-breeding technology will play a major role in improving world food production, as did the green revolution that occurred from the 1960s through the 1980s. We will have to adapt new technologies to the needs of individual countries, industrialized and developing, so that they can effectively adapt and improve their food production without any adverse effects on the environment and biodiversity.

Modern biotechnology is capable of taking plant improvement to new heights with the potential of greatly improving food production. Recently developed successful technologies include, first, tissue culture, in which plants are broken down into cell suspensions and manipulated to regenerate plants, has bypassed some traditional approaches to seed production. Second, anther culture techniques have been successful in creating double haploid populations, greatly reducing the time required to produce cultivars. Third, modern approaches to mutation technology have been successful in creating genetic variation necessary for crop improvement. Fourth, the utilization of molecular marker-assisted selection and other molecular oriented technologies, where various types of DNA fragments, including numerous examples such as restriction fragment length polymorphism (RFLP), simple sequence repeat or microsatellite repeat (SSR), amplified fragment

length polymorphism (AFLP), single nucleotide polymorphism (SNP), diversity array technologies (DArT), and so on, have been and will continue to be linked to value-added traits and have already been successfully used in cultivar improvement programs. Fifth, plant transformation technology, which involves transferring genes from one organism to another bypassing any sexual process, has and will continue to have a significant impact on the adaptation of new cultivars to various biotic and abiotic stresses.

Most of the traditional and newly developed technologies have been and will continue to be adapted to a more land- and labor-intensive form of agriculture improvement. It is clear that organic and subsistence farming applications are neither capable of producing enough to feed nor improve dietary standards of our existing population, let alone the projected increase of 9 billion people by 2040. It has been estimated that organic farming applications are capable of only feeding a world population of approximately 4 billion people (Smil, 2001, 2004; Conner, 2008). The pure organic approach to feeding the world is a theory that simply is not possible and does not take into account the current scale of human suffering from malnutrition and starvation. Embracing social justice for everyone is the only way that humanity can survive and prosper.

Agriculture is certainly capable of feeding the projected world population on approximately the same amount of land currently under production (World Bank World Development Report, 2008). It will take all of our newly developed technologies and plant breeders' skills to achieve the desired goal of satisfying world hunger. Significant progress has been made in advancing our understanding of the world we live in, which can be applied to technology for improving food production. No one knows the direction current research and breeding programs will take, but we can all

assume that any application will have to be determined by economic and social factors. Only the coordinated application of all technologies will sustain the productivity of the lands and maintain our fragile environment.

All crops can be improved by traditional and biotechnological approaches to increase their yield potential. Building adaptable gene complexes from other species and even genera into the crops for the future is something that we have done in the past and must continue to do in the face of global climate change and the world's increasing population. This will require a much larger number of cultivars, with different genetic backgrounds, than in the past. It is very important that we consider improving the world production involving new varieties and management practices, including transgenic crops to the degree it will be possible to predict their impact on wild and weedy crop relatives, and the environment. Such concerns about gene contamination and environmental impacts should be carefully dealt with on an individual crop/environment basis. We must keep firmly in mind that gene complexes from other species and genera have been inserted into most of the world's major crops for more than the past 60 years. For example, Sears (1956) inserted a gene into wheat controlling disease resistance from *Aegilops umbellulata*, which saved wheat from a world rust epidemic. Future crop development will require that, first, we understand the manipulation of gene function and regulation in all crops. Second, we continue to explore and utilize all sources of gene complexes and technology. Third, cropping systems must be characterized to establish the genetic flexibility of various species in diverse ecological contexts, according to their breeding systems, mutation rates, genome recombination properties, and the genomic distribution and function of structural genes. Fourth, we characterize the interface between developing agricultural

ecological dynamics and adaptive ecosystems in order to characterize genome evolution and the potential for gene contamination on an individual crop/location basis. In the past, when modern agriculture competed with the traditional subsistence forms of agriculture, local landrace cultivars were often discarded in favor of the new high-yielding cultivars. Massive efforts have been undertaken to preserve plant diversity, which has resulted in the retention of more old and new diversity in agriculture than existed 50 years ago. National and international seed banks are and will continue to be critically important to agriculture and the maintenance of the world's biodiversity.

The continued long-term health of world food production is one of the foundations to world security. The stable future of humanity, our environment, and our biodiversity are intimately tied to the improvement of crop production. Feeding the masses is clearly the most important challenge facing the world today and in the future.

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Section 1

Perspectives of Gene Flow

1

Potential Environmental Impacts of Transgene Flow in Rice with a Particular View on Herbicide Resistance

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Introduction

The continuous increase in human population and decrease in world arable lands and water resources have challenged the world food security. According to the statement by the Director-General Dr Jacques Diouf of the Food and Agriculture Organization (FAO), United Nations, at the “World Summit on Food Security” held in Rome, Italy, November 16–18, 2008, over 1 billion people are still fighting against hunger. He stressed the urgent need to produce food where the poor and hungry lived and to boost agricultural investment in these regions. To resolve the severe problems of the world, food security will provide a sustainable guarantee to the stabilization of human society. Scientists have proposed that the efficient application of high and new technologies, including transgenic biotechnology, in

agriculture may provide an alternative solution to this problem (Huang et al., 2003). Transgenic biotechnology employs the modern genetic tools to engineer organisms; therefore, this technology provides highly effective and accurate tools for the genetic improvement of crop species. In addition, it can overcome the reproductive isolation when transferring genetic traits between distantly related species, which enable the modification of any crop species almost freely according to human design. Transgenic biotechnology with its new improvement, including cotransformation with multiple transgenes, is considered to be the most promising technology in the twenty-first century (Halpin, 2005).

Since the past 25 years or so, the research and development of transgenic biotechnology has been unprecedented. To date, gene transfer through biotechnology has been successfully achieved in more than 200 plant species, including food crops (e.g., rice, wheat, maize, sorghum, and barley), cash crops (e.g., cotton, soybean, and oilseed rape), vegetables (e.g., tomato, cucumber, leaf mustard, cabbage, and eggplant), and forestation species (*Paulownia*, *Populus*, *Pinus*, and *Eucalyptus*) (Yan, 2001). On the other hand, a large number of functional genes with practical values have been successfully explored in plant genetic engineering to develop transgenic plants. These include high protein content and unique nutritional compounds (Gura, 1999; Ye et al., 2000), disease and insect resistance (Datta et al., 2002; Huang et al., 2005; Bock, 2007), virus resistance (Shepherd et al., 2007; Vanderschuren et al., 2007), herbicide resistance (Lutz et al., 2001; Toyama et al., 2003), as well as salt and drought tolerances (Bahieldin et al., 2005; Tang et al., 2006).

The application of transgenic biotechnology and GM products has had tremendous impacts on world crop production in terms of its potential for poverty alleviation

and solving the problems of malnutrition. By the end of 2009, the estimated global cultivation area of GM crops has exceeded 130 million hectares and generated about US \$52 billion economic gains worldwide (James, 2009). Herbicide-resistant GM crops are among the most successful transgenic products, accounting for more than 65% of total global cultivation area of GM crops. GM crops with other traits also have played important roles in crop production. For example, the cultivation of insect-resistant GM cotton has led to reduced applications of pesticides that can harm human health and agricultural ecosystems (Huang et al., 2005; Brookes and Barfoot, 2009). A considerable decrease in regional outbreaks of cotton boll worms was associated with the extensive cultivation of GM Bt cottons (Wu et al., 2008).

The commercial production of GM crops is important for world food security by enhancing crop production. But, on the other hand, the extensive environmental release and commercial cultivation of GM crop varieties have aroused tremendous biosafety concerns and debates worldwide, including food and feed safety, environmental safety, and long-term availability of biodiversity (for a review, see Lu, 2008). Biosafety issues have already become a crucial factor in constraining the further development of transgenic biotechnology and the wider application of GM products in agriculture. Nowadays, it is not possible to circumvent biosafety issues when discussing the development and application of GM crops (Stewart et al., 2000; Pretty, 2001; Ellstrand, 2001, 2003). Therefore, it is necessary to face the challenge of the biosafety issues aroused by the cultivation of GM crops and try to close the “knowledge gap” by providing solid data from science-based research.

Transgene escape and its potential environmental impacts are among the most debated biosafety issues (Ellstrand et al., 1999; Ellstrand, 2001, 2003; Lu and Snow, 2005;

Wang et al., 2006). What are transgene escape and gene flow? What are the potential environmental impacts caused by transgene flow? Is it possible to minimize transgene flow and to mitigate any negative impacts caused by transgene flow? In this chapter, we shall provide some information to address these questions using herbicide-resistant GM rice as a case study.

Transgene Escape and Its Potential Environmental Impacts

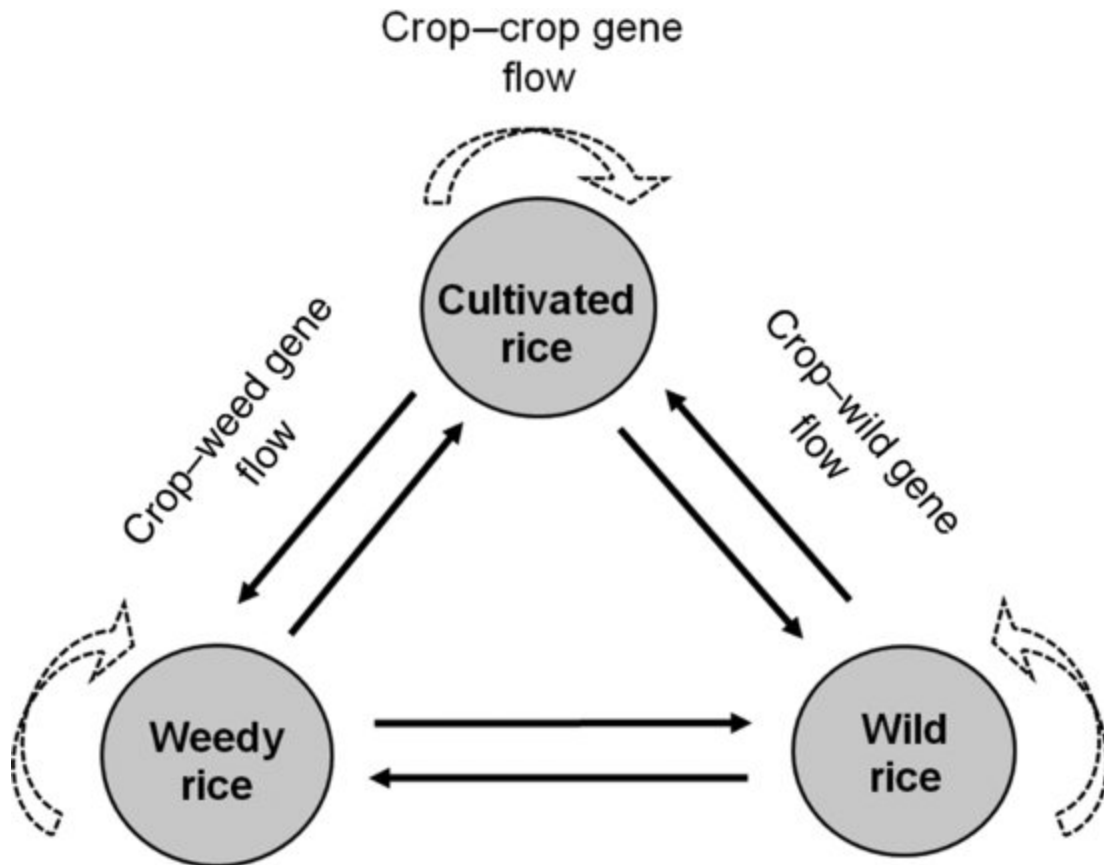
Transgene escape indicates a process in which a transgene(s) moves from a GM crop to its non-GM crop counterparts or to its wild or weedy relatives through gene flow. *Transgene escape will occur and result in potential environmental and biodiversity impacts if transgene flow to non-GM crop varieties and weedy/wild populations is significant.* Because transgene escape is caused by gene flow, it is therefore important to understand what gene flow is and how many types of gene flow there are.

Transgene Flow

Gene flow is a natural process that contributes significantly to the evolution of organisms (Lu, 2008). By a simple definition, gene flow indicates the movement of genetic materials (genes or alleles) from one organism to another. In population genetics, gene flow (also known as gene migration) refers to the transfer of alleles or genes from one population to another (Hartl and Clark, 1989). Through gene flow, different genes or alleles can be transferred among or within biological populations to achieve the exchange and dissemination of genetic information ([Figure 1.1](#)). Theoretically, there are two types of gene flow: (1) vertical gene flow and (2) horizontal gene flow, although the latter is

preferably referred to as horizontal gene transfer (Gogarten and Townsend, 2005).

Figure 1.1 Schematic illustration showing two-directional gene-flow-mediated pollination among cultivated, weedy, and wild rice. Arrows with solid lines indicate gene flow among taxa, and arrows with broken lines indicate gene flow within a taxon.



Horizontal gene transfer occurs only among unrelated species, such as between plants and microorganisms, as well as between different microorganisms (Thomson, 2001). It is recognized as the major force for the genome evolution of some microorganism species. The frequency of horizontal gene transfer is very low (Nielsen et al., 1998). In terms of environmental biosafety, the discussion of horizontal gene transfer is based more on theory than practice, since it has never been shown to occur with transgene outside an experimentally enforced setting, even though this process is

significant in the evolution of microorganisms. Therefore, this chapter will focus only on vertical gene flow that is meaningful in terms of transgene escape and its associated environmental impacts.

Gene flow can maintain plant populations at different spatial distances with a certain degree of genetic relatedness. Reproductive isolation and gene flow function as two major opposite forces in the evolutionary process, with the former promoting speciation or diversity, and the latter maintaining the same genetic identity of a species (Rieseberg et al., 2004). Human activity, such as domestication, serves as a strong isolation force that can produce, by selection and cultivation, a separate population derived from a wild plant species. Therefore, gene flow is a natural process that occurs incessantly and permanently between biologically compatible organisms and to which all genes are subject. In the case of transgene escape, gene flow serves as a medium that moves a transgene from a GM crop to its non-GM counterparts and weedy/wild relatives.

Since gene flow is defined as, for example, in plants, the movement of genes from one plant population to another, any medium such as pollen, seeds, and vegetative organs that can move genes around will lead to gene flow. Typically, there are three avenues for gene flow to be mediated: (1) either by pollen, (2) seed, or (3) vegetative propagules (Andow and Zwahlen, 2006; Lu, 2008).

Pollen-mediated gene flow occurs when pollen grains travel from a plant individual to another individual resulting in fertilization. This process can happen between individuals within the same population or among separate populations. In the latter case, wind, animals, water current, and other factors can serve as media.

Seed-mediated gene flow occurs through the natural dispersal of seeds by animals, wind, water, or other means from one population to another. Animals with long-range

migration habits can transfer seeds over very long distances. Humans can also move seeds intentionally through seed-exchanging and trading within or between geographical regions, which can promote significant amounts of gene flow. The frequencies and patterns of human-influenced seed movement require sociological (seed exchange and distribution) and economic (regional and international trading) analyses and cannot be predicted using only knowledge related to plant biology.

In the case of vegetative-propagule-mediated gene flow, the movement of genes takes place through the natural dispersal of vegetative organs (e.g., tillers, roots, tubers, and rhizomes) of plant species by animals, wind, water, or other means. As for seed-mediated gene flow, the movement of vegetative organs, particularly by animals and humans, is difficult to estimate when based only on plant biology.

Pollen-mediated gene flow will be primarily determined by the intrinsic biological features, particularly the pollination biology of the plant species, such as breeding systems, outcrossing rates, amount of pollen (pollen load) produced by pollen donors, and pollen competition between donors and recipients (Rong et al., 2010). In addition, physical or environmental conditions, such as distances between pollen donors and recipients, the strength and direction of wind, temperature, light intensity, and air humidity, will also influence pollen-mediated gene flow to a great extent (Rong et al., 2010). It is therefore very important to generate such baseline biological and physical data through a science-based approach for the accurate prediction of pollen-mediated gene flow. In agricultural ecosystems, humans can play an important role in seed and vegetative-organ dispersal and migration, as would be the case of seeds or vegetative organs falling on the ground during harvesting and picking, transportation to the processing manufacturers,

and trading at the local, regional, and international level. The intensity and avenues of gene flow in different crop species can vary significantly, depending on annual or perennial characteristics, the capacity for seed dormancy, the longevity of seeds or vegetative propagules during storage (under natural or artificial conditions), differences in breeding (mating) systems, the importance of such crops in national and international markets, and those parts of the crop that are consumed by humans. Given the complexity of gene movement through seeds or vegetative organs, seed-mediated gene flow and vegetative-propagule-mediated gene flow will not be discussed further in this chapter, but it is necessary to point out that these are very important avenues for gene flow in terms of evolutionary processes or GM-related biosafety issues.

Potential Environmental Impacts Associated with Transgene Flow

The most relevant questions relating to transgene flow and its potential biodiversity and environmental impacts should be scientifically addressed and analyzed. This will not only facilitate our objective understanding of the potential biosafety problems caused by transgene flow at various situations but also for the effective assessment and management of transgene flow and its impacts. Such knowledge will guarantee the further development of transgenic biotechnology and promote the safe and sustainable utilization of its products.

The environmental impacts created by transgene escape into different recipients can vary significantly in terms of categories and magnitudes. Transgene escape from GM crops to their non-GM counterparts will have completely different consequences compared with the escape to weedy and wild relative species. Even in the latter case, different

types of transgenic traits will have different effects to wild populations under different environmental conditions and human influences. Therefore, the case-by-case principle should be applied rigidly to assess the environmental impacts from transgene flow, which should be dependent upon the types of recipients (e.g., crops or wild species) that may have acquired the transgenes.

Crop-to-Crop Transgene Flow

The major consequence caused by transgene flow from a GM crop to its non-GM crop counterparts is the “adventitious mixing” of GM and non-GM crop varieties (or so-called “contamination”). If the transgene becomes present in seeds or the derived products of a non-GM crop and is consumed by human or used as animal feed, such a “contamination” may arouse food and feed biosafety concerns, and cause some trading problems between regions or countries. Sometimes, such a “contamination” may even result in legal disputes among different parties. There are already a few examples where the products of nonfood GM crops have been found in mixture with food and feed crops. One of the well-known examples is the Starlink™ GM corn (transformation event CBH-351; Organisation for Economic Cooperation and Development (OECD) unique identifier ACS-ZM004-3) that was only approved for use in animal feed. In 2000, the Bt (Cry9C) toxin from Starlink corn was detected in taco shells, sparking a whole-scale product recall (Heinemann, 2007). In addition, a significant amount of gene flow to non-GM crops has the potential to increase opportunities for subsequent gene movement to weedy or wild rice populations. In these cases, the level of “mixture” or “contamination” from GM crop by gene flow is crucial.

Transgene flow from a GM crop to its non-GM crop counterparts can also lead to the change of genetic diversity in traditional crops. The extensive adoption of GM

crops may lead to rapid losses of traditional crop varieties because of the continuous replacement of the traditional varieties by more commercially advantageous GM varieties. For example, after only a decade of adopting GM cotton, the current cultivation area of insect-resistant GM cotton (Bt) comprises more than 70% of the total cotton cultivation area in China, and more than 65% of the total cotton cultivation area in India (Wu, 2007; James, 2009). In addition, the spread of transgenes from a GM crop variety to non-GM traditional varieties through gene flow may change the integrity of the traditional varieties if the transgenes have a selective advantage. During the process of cultivation and seed production, hybrids containing beneficial transgenes may gradually accumulate unintentionally during selection to ultimately replace the important original genotypes of the traditional varieties (Lu, 2008).

Crop-to-Weed/Wild Transgene Flow

Transgene flow from a GM crop to the weedy and wild relatives of the crop may create invasive weeds if the GM crop that is modified to tolerate herbicides or to resist diseases and pests transfers such traits to wild or weedy relatives *via* gene flow. Crops can also be modified with traits that allow them to grow faster (e.g., by expressing a specific growth hormone), reproduce more (e.g., by enhancing seed production), and live in new types of habitats (e.g., by enhancing drought and cold tolerance). The potential environmental impacts caused by crop-to-weedy or crop-to-wild gene flow need to be determined in the long term. There are still many biological mechanisms underlying the process of gene flow and fitness change to be understood. The following are only some of the hypothesized or predicted consequences of crop-to-wild gene flow that are commonly discussed and debated