Consequences of gene flow from genetically engineered crops

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Out crossing is ubiquitous in both self and cross-pollinated crops. The possibilities of genetically engineered (GE) crops out crossing with other crop cultivars of modern agriculture, traditional land races, and sympatric wild, weedy, related species, and consequent gene flow have emerged as a reality based on scientific data. The problem is not unique to GE crops. It was associated with all new crop cultivars developed using traditional breeding methods. Introduction of GE crops has drawn the attention to this issue. Experimental data on the long-term consequences of gene flow on the recipient populations are not available. Such data can be obtained by investigating land races, and natural stands of wild, related rice and sorghum species in the neighborhoods of areas where semi-dwarf, insect, and disease resistant cultivars/hybrids have been grown since mid 1960s. Associated DNA-based markers can be used to estimate the extent of introgression from the crop cultivars. Consequences of trangene(s) flow can be investigated by hybridizing the GE crops to land races, and wild weedy relatives of the crop, in parallel, to the agronomic evaluation.

Various apprehensions have been articulated against the cultivation of crop plants with genetically enhanced traits developed using recombinant DNA techniques. Such crops have been referred to as genetically modified (GM), transgenic, genetically engineered plants (GEPs) or plants with novel traits (PNTs). They are also called living modified organisms (LMOs). Plants engineered with pesticide-producing traits are referred to as plant-incorporated protectants (PIPs). Here, crops developed using r-DNA techniques are referred to as genetically engineered (GE), as this clearly defines the technique used for the improvement. The biosafety and environmental concerns have caused a serious setback for the GE crops in Europe, and have delayed their approval for commercial cultivation in many other countries. There is a large amount of information on the environmental concerns with respect to cultivation of GE crops, going back to the issues first raised by Rissler and Mellon[1]. Literature on biosafety of GE crops till 1997 was reviewed by us[2]. Subsequently, these issues have been addressed by national academies[3] and other researchers[4,5]. The regulatory agencies in different countries, after carefully weighing the possible risk and the benefits, have cleared a number of transgenic crops for commercial cultivation in the respective countries. The results show that the early concerns mainly reflected 'fear of the unknown', and most of the anticipated adverse effects have not been observed. During the year 2001 transgenic crops were grown globally on an area exceeding 52 m ha, cumulative area between 1995 and 2001 exceeding 75 m ha (ref. 6).

However, the possibilities of such crops out crossing with the other crop cultivars of modern agriculture, land races, 'traditional' populations, and wild or weedy relatives of the crop plants have emerged as a reality based on scientific data.

Out crossing is ubiquitous in both self and cross-pollinated species. The problem of out crossing is not new or unique to the GE crops; it has been there for all new crop cultivars. Here, we examine the possible consequences of transgene introgression in crop cultivars, land races, and wild and weedy relatives. It should be clearly understood that generalizations are not possible in this area, and each GE crop needs to be investigated on a case-by-case basis, considering the distribution of the possible recipients of the transgene(s) in the GE crop-growing area. In the Indian context where only GE cotton has been approved for commercial cultivation so far, experimental approaches to obtain meaningful, scientific data are suggested. There is urgent need to generate information for situations where wild relatives and GE crops are likely to be sympatric, for example, rice and sorghum. Experimental data would facilitate decision making in the regulatory process.

Crossing between sympatric plant species and gene flow between them at low probabilities is well known[7–10]. GE crops are not expected to be different unless associated with some alterations in the floral characters. Plant breeders are always conscious of out crossing, even in highly self-pollinated crops, and appropriate methods have been developed to maintain the purity of the cultivars.

The possibilities of out crossing, and consequent gene flow, defined as the movement of genes from one population to another[11], have been exploited by the opponents of the r-DNA technology. They infer that the introgression of transgenes will contaminate (pollute), reduce or destroy biodiversity. Such arguments influence public opinion. In the absence of hard scientific data, the problem of gene flow has drawn the attention of scientists working in different disciplines of plant sciences. Current interest in the area is evident from two international conferences[12,13] in the last few months focused on gene flow. It is also one of the major research areas targeted by the Biotechnology Risk Assessment Research Grant Programme of the United States Department of Agriculture.

The topic of gene flow also became a highly emotional issue after the publication of a paper by Quist and Chapela[14] in *Nature*, showing the presence of DNA sequences associated with transgenic maize in the native, open-pollinated land races of maize in Mexico. The publication was followed by severe criticism from different experts in the scientific community that led *Nature* to retract the paper[15] (also see ref. 16). The commercial and political implications of gene flow have recently emerged in Zimbabwe, Zambia and some other countries in the southern part of Africa. There is acute shortage of maize – their principal food crop – due to severe drought, yet these countries have refused maize of US origin donated under the World Food Programme. About 30% of the maize grown in the USA is from GE hybrids and the produce is not segregated. Hence, the donated maize originating from USA cannot be certified as GE-free. There are apprehensions that part of

the grain received as food will be planted by the farmers to raise the crop during the next season. Pollen from the GE plants would contaminate the local maize. Under the new legislation of the European Union (EU), the GE seed level should be less than one per cent. The governments in these African countries fear that the probable gene flow into the local maize would be detrimental for its export to the EU in future.

Gene flow can result from the dispersal of seeds as independent propagules or the pollen. Dispersal of seeds leading to volunteer plants on the field bunds and roadside, intentional or unintended use of grains obtained as food can also contribute to gene flow. While the genes introduced in the chloroplast and nuclear genomes can move only through the seeds, gene flow through pollen is considered significant for transgenes incorporated in the nuclear genome. Out crossing depends on a number of biological and environmental factors listed (see Table 1). Frequency of out crossing is estimated using different methods; best estimates are obtained using trap plants with recessive genes grown at various distances from crop cultivars or genetic stock with known, easily identifiable, dominant markers. Cultivaral differences in the extent of out crossing are known. Intra-species (or between different crop cultivars in the agricultural context) gene flow to some extent is ubiquitous even in highly self-pollinated crops. As a part of the regulatory process, out crossing through pollen from GE crops is investigated before its approval for commercial cultivation. However, such studies have mainly focused on the distance to which pollen from transgenic crops can effectively pollinate the trap plants. Further detailed studies have been reported from Brassica species[17,18], Raphanus sativus[19], sugar beet[20] and peas[21]. The possible consequences of trangene flow into other crop cultivars of modern agriculture, land races and wild relatives have not been investigated, and therefore, it is important to obtain such data.

Rice is of primary concern in India as the foothills of the Himalayas and associated mountain ranges in the eastern part of the country are believed to be the area of its origin[22], and Jeypore tract in Orissa and Chattisgarh region are well recognized for large biodiversity in cultivated and related species of rice. Swarms of hybrids between cultivated rice (Oryza sativa) and wild species of Oryza are reported in these areas. Currently, there are intensive research programmes for the development of GE rice in the country (Table 2) and in China. Some of these may be ready for commercialization in the next few years. Meanwhile, information on long-term consequences of gene flow and gene introgression can be gathered using the semi-dwarf rice cultivars as model. Semi-dwarf, high-yielding rice cultivars have been grown since mid 1960s, along with tall traditional varieties and land races; in many areas, wild, perennial and annual species of genus Oryza are sympatric. The consequences of introgression of the transgene(s) into wild relatives can be investigated by crossing homozygous transgenics to wild, related species. Much more data will be required for approval of plants having human genes, or genes for therapeutic proteins from the Genetic Engineering Approval Committee. Recently, transfer and expression of human lysozyme gene in rice has been reported[23].

Gene flow into other cultivars of modern agriculture

With two per cent out crossing between rice cultivars, the same percentage of grains harvested from the neighbouring crop could be heterozygous for the transgene. If part of the harvest is used for planting the next crop, the same percentage of plants will be heterozygous, and in the next generation they would segregate: 0.5% each of homozygotes (TT) and with no transgene (_ _), and 1% heterozygotes (T_). In the absence of any selective advantage, this frequency would be maintained. Repeated events of out crossing from GE crop could increase the number of plants with transgene. However, if an agronomically desirable transgene provides a selective advantage to the plants carrying the transgene and increases its fitness (reproductive ability), their numbers in the population would increase. Thus, the frequency of the transgene would depend on its selective advantage. In rare events of single gene heterosis, the fitness of the heterozygote may be significantly higher and consequently, the spread would be much faster. In the new genetic background, the chances of 'gene silencing'[24] cannot be entirely ruled out.

Table 1. Factors determining out crossing and gene flow in plants

Presence of receptive stigmas of sexually compatible cultivars, traditional cultivars, land races, wild and weedy relatives within the viable pollen transmission range.

Flowering phenology of the above-mentioned species. The overlap of the flowering period enhances the chances of out crossing.

Viable pollen movement distance determined by pollination mechanism and viability of the pollen.

Pollination mechanism – self or cross-pollinated.

Means of pollination in wild and weedy related species.

Wind or insect?
In case of wind pollination, factors like wind direction and speed determine the distance travelled.
In insect pollinated plants, whether the crop and related species are visited by the same insect pollinators. Distance travelled by the insect pollinator.

Viability is determined by pollen characteristics and environmental factors such as temperature, relative humidity, etc.

The outcome of pollen deposition on receptive stigma of related species is dependent on several factors – pollen tube growth, and length of style. The pollen tube must cover to reach the ovule for fertilization.

Outcome of fertilization, seed set and viability of such hybrid seeds, their germination, growth and fertility would further depend on the number and size of chromosomes in the two species. Sterile hybrids not capable of sexual reproduction can survive through vegetative propagation in crops like rice.

Polyploidization of sterile hybrids or repeated natural backcrossing to one of the species can lead to natural selection of fertile hybrids.

Table 2. Current research programmes for gene transfer to rice in India

Insect resistance

    Bacillus thuringiensis genes *cry1A*(*b*), *cry1A*(*c*) and their fusion products
    Synthetic *cry1Ac* gene
    Cry9A(*a*)
    Proteinase inhibitor apoprotein which inhibits gut proteinases of several Lepidopteran insects
    Potato protease inhibitor 2 (*Pin-II*) for insect resistance
    Garlic leaf lectin – *Allium sativum* agglutinin of leaf (ASAL)
    Snow drop (*Gallanthus nivalis*) lectin (agglutinin) (*GNA*) gene.

Disease resistance

    Xa21 gene from *Oryza longistaminata* for bacterial blight resistance
    Thaumatin like protein (*tlp*)
    Beta 1,3-glucanase gene
    Rice chitinase (*chi11*) gene
    Trichoderma chitinase gene
    Arabidopsis NPR1 gene which regulates salicylic acid-mediated systemic acquired resistance.

Virus resistance

    Rice tungro bacilliform virus (RTBV) promoter
    Rice tungro spherical virus (RTSV) coat protein.

Stress resistance

    Barley late embryogenesis abundant protein (Lea 3)
    Mothbean pyroline-5-carboxylate synthetase (*P5CS-129-A*) gene.
    Transit peptide plus manitol 1-phosphate dehydrogenase (*TP-mt1DH*) gene
    Choline oxidase (*codA*) gene from *Arthobacter globiformis* for glycine-betaine accumulation associated with stress tolerance
    Inositol synthase gene from wild, halophytic rice *Porteresis coarcata*
    Genes involved in polyamine biosynthesis
        Mouse ornithine decarboxylase (*odc*) in sense and antisense
        Oats arginine decarboxylase (*adc*)
        Human adenosyl methionine decarboxylase (*samdc*)
        Datura spermidine synthase (*spd syn*)
    Annexin gene of *Arabidopsis* in sense and antisense.

Male sterility

    T-urf-13 – a maize gene associated with Texas cytoplasmic male sterility
    RASP – rice anther-specific promoter.

Multiple genes

    Five minimal transgene cassette hygromycin phosphotransferase (*hpt*), phosphinothricin transferase (*bar*), beta-glucuronidase (*gusA*), luciferase (*luc-1*) and anthrinalate synthase (*as*) genes.

Most of these transgenics are in early generations.

Information based on Abstracts of 8th National Rice Biotechnology Network Meeting held during 21–25 October 2001 at Aurangabad and organized by MAHYCO Research Foundation, Mumbai.

Since the GE cultivars approved for commercial cultivation would have been examined for biosafety in the regulatory process, incorporation of the transgene into another cultivar would not have any adverse environmental effect. Seed replacement, as practised in modern agriculture, would eliminate the transgene. In the extreme scenario, it is possible that only the homozygotes for the transgene survive and all other plants fail to produce any seeds. Such an event could produce a new GM cultivar, raising questions of the misuse of the intellectual property of the company that originally released the GE cultivar. A farmer in Canada claimed that resistance to the herbicide in his crop was introduced through pollen flow from the neighbouring fields[25], though his argument was not accepted in the court.

Land races

Land races are not as uniform and homozygous as the released cultivars resulting from intensive breeding. Mostly, they are a mixture of several different genotypes, and genetic exchanges among constituent genotypes is common. Pollen flow from GE cultivars will have effects similar to those on modern cultivars, except that the expression of transgene can be different depending on the genotype. Introgression of the transgene would have similar effect on the land races; it could increase, decrease or have no effect on selective advantage. Gene flow would enlarge the diversity of the land races and should not be a matter of concern. These expectations can be easily verified by field experiments, in parallel, with field evaluation of the GE cultivars. Moreover, the land races have no place in modern, high-productivity farming and should be conserved *in situ* on a limited scale, and in the gene banks.

Gene flow among crops and their wild/weedy related species

Among the major cereal crops grown in the country, only rice and sorghum have their wild related species growing in the same geographical regions. Cucurbits, *Vigna* species, *Cajanus* and some Solanaceous crops also have their related species in the country. Various *Brassica* species having different genomic constitution and ploidy levels are grown. Ellstrand[9,10] has stressed on the importance of crop to wild species gene flow in the context of large-scale cultivation of GE crops. The impact of immigrant alleles at population, community and ecosystem levels has been emphasized. The extent of inter-species hybridization between crops and their local wild species, except in rice and sorghum, is not known. *Oryza nivara*, having the same genome as *O. sativa*, is easily hybridized; but other species with non-homologous genomes are difficult to hybridize. Such hybrids were obtained using

O. sativa as female and embryo rescue[26]. The ecological consequences of gene flow of agronomically useful transgenes to wild relatives are not known, except for herbicide resistance. It is therefore important to introduce these genes into wild, related species by hybridization or direct transformation of the wild species to obtain empirical data. Johnson grass (*Sorghum halepense*) is sympatric in most sorghum (*Sorghum bicolor*)-growing areas and natural hybridization between the two species is reported[27].

Wild and weedy species show greater diversity than the land races; consequence of gene flow to sexually compatible relatives will be similar to those of the land races. Inter-species outcrossing and gene flow is dependent on several independent factors – pollination mechanism, differences in genome, ploidy level, chromosome number and size, seed set, viability and vigour of the hybrid plants would vary following out crossing with not-easy-to-cross species. The worst scenario would lead to the introgression of the transgenic trait into the wild, weedy species. This may not cause any serious problem in most disease and pest resistance transgenes listed in Table 2, as many of the wild relatives are well-known sources of such resistance genes. However, incorporation of abiotic resistance such as herbicide resistance could have serious consequences in weedy species that grow along with the crop in the same agricultural field. Some perennial wild-rice species propagate vegetatively, and may not depend on seed production for their spread. Increased seed production in wild sunflower as a result of herbivore resistance due to transfer of *Bt* toxin gene has been observed[28]. Multiple herbicide resistance in volunteer *Brassica napus* plants has been attributed to gene flow from different herbicide-resistant *B. napus* cultivars[29]. Similarly, increase in drought tolerance of a wild species can enhance its fitness over the competing species, and eventually alter the species distribution in natural ecosystems. Increased nutritional value of wild species could lead to enhanced fitness of the foraging insect species. The question whether these ecological changes would be different from what goes on in nature due to natural causes such as floods, drought, landslides or human interventions, grazing of animals, construction of buildings, etc. needs to be addressed based on empirical data.

Opportunities for collecting empirical data

In the absence of hard data on gene flow from GE crops and its long-term consequences on natural biodiversity, best approximations can be obtained by monitoring natural stands of wild rice and sorghum species close to areas of extensive cultivation of semi-dwarf, insect and disease-resistant cultivars and hybrids of these crops developed by conventional methods of breeding. In some areas, semi-dwarf types have been under cultivation for over 30 years. The frequency of semi-dwarfs in natural stands and their progeny testing itself can provide some realistic estimates on out crossing and consequences of gene flow from cultivated species. Various DNA-based methods currently used for polymorphism studies can provide information on the possible introgression of markers associated with the semi-dwarf genotypes and possible long-term consequences of gene flow on biodiversity. Such experiments though difficult, are doable. As pointed out in our earlier publication[2], in future, similar questions on multiple transgenes introduced into commercial cultivars will be raised. Three different genes have been transferred in the 'golden rice' and recently, an attempt has been made to transfer and express nine transgenes into rice[30].

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