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Transgenic Technology and Crop Improvement - A Reveiw

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A transgenic crop plant contains a gene or genes which have been artificially inserted instead of the plant acquiring them through pollination. The inserted gene sequence (known as the transgene) may come from another unrelated plant, or from a completely different species: transgenic Bt corn, for example, which produces its own insecticide, contains a gene from a bacterium. Plants containing transgenes are often called genetically modified or GM crops, although in reality all crops have been genetically modified from their original wild state by domestication, selection and controlled breeding over long periods of time. On this web site we will use the term transgenic to describe a crop plant which has transgenes inserted.

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Transgenic plants possess a gene or genes that have been transferred from a different species. Although DNA of another species can be integrated in a plant genome by natural processes, the term "transgenic plants" refers to plants created in a laboratory using recombinant DNA technology. The aim is to design plants with specific characteristics by artificial insertion of genes from other species or sometimes entirely different kingdoms.

Varieties containing genes of two distinct plant species are frequently created by classical breeders who deliberately force hybridization between distinct plant species when carrying out interspecific or intergeneric wide crosses with the intention of developing disease resistant crop varieties. Classical plant breeders use a number of in vitro techniques such as protoplast fusion, embryo rescue or mutagenesis to generate diversity and produce plants that would not exist in nature.

Such traditional techniques (used since about 1930 on) have never been controversial, or been given wide publicity except among professional biologists, and have allowed crop breeders to develop varieties of basic food crop, wheat in particular, which resist devastating plant diseases such as rusts. Hope is one such wheat variety bred by E. S. McFadden with a gene from a wild grass. Hope saved American wheat growers from devastating stem rust outbreaks in the 1930s. Methods used in traditional breeding that generate plants with DNA from two species by non-

recombinant methods are widely familiar to professional plant scientists, and serve important roles in securing a sustainable future for agriculture by protecting crops from pests and helping land and water to be used more efficiently.

A plant breeder tries to assemble a combination of genes in a crop plant which will make it as useful and productive as possible. Depending on where and for what purpose the plant is grown, desirable genes may provide features such as higher yield or improved quality, pest or disease resistance, or tolerance to heat, cold and drought. Combining the best genes in one plant is a long and difficult process, especially as traditional plant breeding has been limited to artificially crossing plants within the same species or with closely related species to bring different genes together. For example, a gene for protein in soybean could not be transferred to a completely different crop such as corn using traditional techniques. Transgenic technology enables plant breeders to bring together in one plant useful genes from a wide range of living sources, not just from within the crop species or from closely related plants. This technology provides the means for identifying and isolating genes controlling specific characteristics in one kind of organism, and for moving copies of those genes into another quite different organism, which will then also have those characteristics. This powerful tool enables plant breeders to do what they have always done - generate more useful and productive crop varieties containing new combinations of genes - but it expands the possibilities beyond the limitations imposed by traditional cross-pollination and selection techniques.

Natural movements of genes between species

Natural movement of genes between species, often called horizontal gene transfer or lateral gene transfer, can occur because of gene transfer mediated by natural processes.

This natural gene movement between species has been widely detected during genetic investigation of various natural mobile genetic elements, such as transposons, and retrotransposons that naturally translocate to new sites in a genome, and often move to new species over an evolutionary time scale. There are many types of natural mobile DNAs, and they have been detected abundantly in food crops such as rice.

These various mobile genes play a major role in dynamic changes to chromosomes during evolution and have often been given whimsical names, such as Mariner, Hobo, Trans-Siberian Express (Transib), Osmar, Helitron, Sleeping Princess, MITE and MULE, to emphasize their mobile and transient behavior.

Genetically mobile DNA contitututes a major fraction of the DNA of many plants, and the natural dynamic changes to crop plant chromosomes caused by this natural transgenic DNA mimics many of the features of plant genetic engineering currently pursued in the laboratory, such as using transposons as a genetic tool, and molecular cloning. See also transposon, retrotransposon, integron, provirus, endogenous retrovirus, heterosis, Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in maize.

There is new scientific literature about natural transgenic events in plants, through movement of natural mobile DNAs called MULEs between rice and Setaria millet.

It is becoming clear that natural rearrangements of DNA and horizontal gene transfer play a pervasive role in natural evolution. Importantly many, if not most, flowering plants evolved by transgenesis - that is, the creation of natural interspecies hybrids in which chromosome sets from different plant species were added together. There is also the long and rich history of interspecies cross-breeding with traditional methods.

Deliberate creation of transgenic plants during breeding

Production of transgenic plants in wide-crosses by plant breeders has been a vital aspect of conventional plant breeding for about a century. Without it, security of our food supply against losses caused by crop pests such as rusts and mildews would be severely compromised. The first historically recorded interspecies transgenic cereal hybrid was actually between wheat and rye (Wilson, 1876).

In the 20th century, the introduction of alien germplasm into common foods was repeatedly achieved by traditional crop breeders by artificially overcoming fertility barriers. Novel genetic rearrangements of plant chromosomes, such as insertion of large blocks of rye (Secale) genes into wheat chromosomes (‘translocations’),

has also been exploited widely for many decades.

By the late 1930s with the introduction of colchicine, perennial grasses were being hybridized with wheat with the aim of transferring disease resistance and perenniality into annual crops, and large-scale practical use of hybrids was well established, leading on to development of Triticosecale and other new transgenic cereal crops. In 1985 Plant Genetic Systems (Ghent, Belgium), founded by Marc Van Montagu and Jeff Schell, was the first company to develop genetically engineered (tobacco) plants with insect tolerance by expressing genes encoding for insecticidal proteins from Bacillus thuringiensis (Bt).

Transgenic rice plants are tolerent to rice tungro virus replication and disease

Rice tungro disease (RTD) accounts for ≈ $1.5 billion annual loss in rice production worldwide (1, 2), and epidemics of tungro disease in the last century caused famines and great loss of human life (1–5). RTD results from coinfection by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Typical disease symptoms include stunting and discoloration of infected plants, reduced tillering, and small and/or sterile panicles. When plants are infected in the early seedling stage, yield losses can be as much as 100%[4,6,7]. The disease is transmitted by green leafhoppers (GLH) (Nephotettix virescens) in a semipersistent manner. RTBV is the causative reagent of RTD symptoms, whereas RTSV is required for disease transmission[8]. Disease-tolerant cultivars have thus far been selected by plant breeders; many such lines rely on resistance to insect transmission[5]. Pathogen-derived resistance against RTD was reported as being only partially effective, although recent reports involving an RNAi construct are encouraging[5,9]. Nevertheless, because of the limitations of current breeding programs and disease management, RTD remains a serious threat to rice production in regions of South and Southeast Asia.

RTBV is a plant pararetrovirus with a circular 8-kb dsDNA genome[10]. Transcription of the RTBV DNA genome is regulated by a promoter located in the intergenic region between ORF IV and ORF I. RTBV accumulates in vascular tissues and activity of the RTBV promoter is largely restricted to vascular tissues. Several cis-acting regulatory elements were identified as contributing to the regulation of expression of this promoter[11,12], including a unique box II element located immediately upstream of the TATA box[12, 13]. Two basic leucine zipper (bZIP)-type rice proteins, RF2a and RF2b, were shown to interact with BoxII and activate transcription from the RTBV promoter in vitro and in vivo (13–15). RF2a and RF2b are also important for rice development, and transgenic rice lines in which their levels were reduced by (–)sense RNA exhibited phenotypes that, in part, resembled the symptoms of RTD[14,15]. In addition, constitutive expression of a dominant negative mutant of RF2a in transgenic tobacco plants caused severe stunting[16]. These observations led us to hypothesize that RTBV causes redistribution of important host transcription factors, including RF2a and RF2b, to favor transcription of the RTBV viral promoter over host genes. We propose that favoring the RTBV promoter may perturb the expression of genes that are important for plant growth and development and/or disease defense resulting in development of disease symptoms. In this study we show that overexpression of RF2a and RF2b in transgenic rice plants reduces virus accumulation and gene expression and leads to tolerance to RTBV.

Genetically engineered plants

The intentional creation of transgenic plants by laboratory based recombinant DNA methods is more recent (from the mid-70s on) and has been a controversial development in the field of biotechnology opposed vigorously by many NGOs, and several governments, particularly within the European Community. These transgenic recombinant plants (biotech crops, modern transgenics) are transforming agriculture in those regions that have allowed farmers to adopt them, and the area sown to these crops has continued to grow globally in every years since their first introduction in 1996

Transgenic recombinant plants are generated in a laboratory by adding one or more genes to a plant's genome,and the techniques frequently called transformation. Transformation is usually achieved using gold particle bombardment or through the process of Horizontal gene transfer using a soil bacterium, *Agrobacterium tumefaciens,* carrying an

engineered plasmid vector, or carrier of selected extra genes.

Transgenic recombinant plants are identified as a class of genetically modified organism(GMO); usually only transgenic plants created by direct DNA manipulation are given much attention in public discussions.

Transgenic plants have been deliberately developed for a variety of reasons: longer shelf life, disease resistance, herbicide resistance, pest resistance, non-biological stress resistances, such as to drought or nitrogen starvation, and nutritional improvement (see Golden rice). The first modern recombinant crop approved for sale in the US, in 1994, was the FlavrSavr tomato, which was intended to have a longer shelf life. The first conventional transgenic cereal created by scientific breeders was actually a hybrid between wheat and rye in 1876 (Wilson, 1876). The first transgenic cereal may have been wheat, which itself is a natural transgenic plant derived from at least three different parenteral species.

Genetically modified organisms were prior to the coming of the commercially viable crops as the FlavrSavr tomato, only strictly grown indoors (in laboratories). However, after the introduction of the Flavr Savr tomato, certain GMO-crops as GMO-soy and GMO-corn where in the USA being grown outdoors on large scales.

Commercial factors, especially high regulatory and research costs, have so far restricted modern transgenic crop varieties to major traded commodity crops, but recently R&D projects to enhance crops that are locally important in developing counties are being pursued, such as insect protected cow-pea for Africa and insect protected Brinjal eggplant for India.

Transgenic plants have been used for bioremediation of contaminated soils. Mercury, selenium and organic pollutants such as polychlorinated biphenyls (PCBs) have been removed from soils by transgenic plants containing genes for bacterial enzymes.

Regulation of transgenic plants

The examples and perspective in this article may not represent a worldwide view of the subject. Please improve this article or discuss the issue on the talk page.

In the United States the Coordinated Framework for Regulation of Biotechnology governs the regulation of transgenic organisms, including plants. The three agencies involved are: USDA Animal and Plant Health Inspection Service - who state that

The Biotechnology Regulatory Services (BRS) program of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is responsible for regulating the introduction (importation, interstate movement, and field release) of genetically engineered (GE) organisms that may pose a plant pest risk. BRS exercises this authority through APHIS regulations in Title 7, Code of Federal Regulations, Part 340 under the Plant Protection Act of 2000. APHIS protects agriculture and the environment by ensuring that biotechnology is developed and used in a safe manner. Through a strong regulatory framework, BRS ensures the safe and confined introduction of new GE plants with significant safeguards to prevent the accidental release of any GE material. APHIS has regulated the biotechnology industry since 1987 and has authorized more than 10,000 field tests of GE organisms. In order to emphasize the importance of the program, APHIS established BRS in August 2002 by combining units within the agency that dealt with the regulation of biotechnology. Biotechnology, Federal Regulation, and the U.S. Department of Agriculture, February 2006, USDA-APHIS Fact Sheet

United States Environmental Protection Agency - evaluates potential environmental impacts, especially for genes which encode for pesticide production

DHHS, Food and Drug Administration (FDA) - evaluates human health risk if the plant is intended for human consumption

"Cisgenic" plants

The term cisgenic is now being introduced by some plant producers to refer to artificial genetic transfers that could theoretically have been replicated by conventional crossbreeding methods. Producers argue that "cisgenically" produced organisms do not have the same degree of novelty as "transgenic" organisms, and involve no environmental issues that are not already present in conventional crossbreeding. It is argued[8] that "cisgenic" modification is useful for plants that are difficult to crossbreed predictably by conventional means (such as

Is the transgenic plant capable of growing outside a cultivated area?

Can the transgenic plant pass its genes to a local wild species, and are the offspring also fertile?

Does the introduction of the transgene confer a selective advantage to the plant or to hybrids in the wild?

Many domesticated plants can mate and hybridise with wild relatives when they are grown in proximity, and whatever genes the cultivated plant had can then be passed to the hybrid. This applies equally to transgenic plants and conventionally bred plants, as in either case there are advantageous genes that may have negative consequences to an ecosystem upon release. This is normally not a significant concern, despite fears over 'mutant superweeds' overgrowing local wildlife: although hybrid plants are far from uncommon, in most cases these hybrids are not fertile due to polyploidy, and will not multiply or persist long after the original domestic plant is removed from the environment. However, this does not negate the possibility of a negative impact.

In some cases, the pollen from a domestic plant may travel many miles on the wind before fertilising another plant. This can make it difficult to assess the potential harm of crossbreeding; many of the relevant hybrids are far away from the test site. Among the solutions under study for this concern are systems designed to prevent transfer of transgenes, such as Terminator Technology, and the genetic transformation of the chloroplast only, so that only the seed of the transgenic plant would bear the transgene. With regard to the former, there is some controversy that the technologies may be inequitable and might force dependence upon producers for valid seed in the case of poor farmers, whereas the latter has no such concern but has technical constraints that still need to be overcome. Solutions are being developed by EU funded research programmes such as Co-Extra and Transcontainer.

There are at least three possible avenues of hybridization leading to escape of a transgene:

- Hybridization with non-transgenic crop plants of the same species and variety.
- Hybridization with wild plants of the same species.
- Hybridization with wild plants of closely related species, usually of the same genus.

However, there are a number of factors which must be present for hybrids to be created.

The transgenic plants must be close enough to the wild species for the pollen to reach the wild plants.

- The wild and transgenic plants must flower at the same time.
- The wild and transgenic plants must be genetically compatible.

In order to persist, these hybrid offspring:

- Must be viable, and fertile.
- Must carry the transgene.

Studies suggest that a possible escape route for transgenic plants will be through hybridization with wild plants of related species.

It is known that some crop plants have been found to hybridize with wild counterparts.

It is understood, as a basic part of population genetics, that the spread of a transgene in a wild population will be directly related to the fitness effects of the gene in addition to the rate of influx of the gene to the population. Advantageous genes will spread rapidly, neutral genes will spread with genetic drift, and disadvantageous genes will only spread if there is a constant influx.

The ecological effects of transgenes are not known, but it is generally accepted that only genes which improve fitness in relation to abiotic factors would give hybrid plants sufficient advantages to become weedy or invasive. Abiotic factors are parts of the ecosystem which are not alive, such as climate, salt and mineral content, and temperature. Genes improving fitness in relation to biotic factors could disturb the (sometimes fragile) balance of an ecosystem. For instance, a wild plant receiving a pest resistance gene from a transgenic plant might become resistant to one of its natural pests, say, a beetle. This could allow the plant to increase in frequency, while at the same time animals higher up in the food chain, which are at least partly dependent on that beetle as food source, might decrease in abundance. However, the exact consequences of a transgene with a selective advantage in the natural environment are almost impossible to predict reliably.

It is also important to refer to the demanding actions that government of developing countries had been building up among the last

decades.

Agricultural impact of transgenic plants

Outcrossing of transgenic plants not only poses potential environmental risks, it may also trouble farmers and food producers. Many countries have different legislations for transgenic and conventional plants as well as the derived food and feed, and consumers demand the freedom of choice to buy GM-derived or conventional products. Therefore, farmers and producers must separate both production chains. This requires coexistence measures on the field level as well as traceability measures throughout the whole food and feed processing chain. Research projects such as Co-Extra, SIGMEA and Transcontainer investigate how farmers can avoid outcrossing and mixing of transgenic and non-transgenic crops, and how processors can ensure and verify the separation of both production chains.

REFERENCES

1. Hull R., Matthews' Plant Virology (Academic, London) 2002.
2. Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP., Signaling in plant–microbe interactions. *Science* 1997; **276**:726-733.
3. Herdt R., in Rice Biotechnology, ed Khush GT (CABI, Wallingford, UK), 1991; 19-54.
4. Azzam O, Chancellor T., The biology, epidemiology, and management of rice tungro disease in *Asia. Plant Dis* 2002; **86**: 88-100.
5. Mew T, Leung H, Savary S, Cruz C, Leach J., Looking ahead in rice disease research and management. *Crit Rev Plant Sci* 2004; **23**: 103-127.
6. John VT, Ghosh A., in Assessment of Crop Losses Due to Pests and Diseases, eds Govindu HC, Veerash GK, Walker PT, Jenkin JF (University of Agricultural Sciences, Bangalore), 1980; 225-231.
7. Periasamy M, Niazi FR, Malathi VG., Multiplex RT-PCR, a novel technique for the simultaneous detection of the DNA and RNA viruses causing rice tungro disease. *J Virol Methods* 2006; **134**: 230-236.
8. Dasgupta I, *et al.*, Rice tungro bacilliform virus DNA independently infects rice after Agrobacterium-mediated transfer. *J Gen Virol* 1991; **72**: 1215-1221.
9. Tyagi H, Rajasubramaniam S, Rajam MV, Dasgupta I., RNA-interference in rice against Rice tungro bacilliform virus results in its decreased accumulation in inoculated rice plants. *Transgenic Res* 2008; **17**: 897-904.
10. Qu RD, *et al.,* Characterization of the genome of rice tungro bacilliform virus: Comparison with Commelina yellow mottle virus and caulimoviruses. *Virology* 1991; **185**: 354-364.
11. Yin Y, Chen L, Beachy R., Promoter elements required for phloem-specific gene expression from the RTBV promoter in rice. *Plant J* 1997; **12**: 1179-1188.
12. He X, Hohn T, Futterer J., Transcriptional activation of the rice tungro bacilliform virus gene is critically dependent on an activator element located immediately upstream of the TATA box. *J Biol Chem* 2000; **27**5: 11799–11808.
13. Dai S, Zhang Z, Bick J, Beachy RN., Essential role of Box II cis element and cognate host factors in regulating the promoter of rice tungro bacilliform virus. *J Gen Virol 2006*; **87**: 715-722.
14. Yin Y, Zhu Q, Dai S, Lamb C, Beachy RN., RF2a, a bZIP transcriptional activator of the phloem-specific rice tungro bacilliform virus promoter, functions in vascular development. EMBO J 1997; **16**: 5247-5259.
15. Dai S, Zhang Z, Chen S, Beachy RN., RF2b, a rice bZIP transcription activator, interacts with RF2a and is involved in symptom development of rice tungro disease. *Proc Natl Acad Sci USA* 2004; **101**: 687-692.
16. Petruccelli S, *et al.,* Transcription factor RF2a alters expression of the rice tungro bacilliform virus promoter in transgenic tobacco plants. *Proc Natl Acad Sci* USA 2001; **98**: 7635-7640.
17. Yang NS, Russell D., Maize sucrose synthase-1 promoter directs phloem cell-specific expression of Gus gene in transgenic tobacco plants. *Proc Natl Acad Sci* USA 1990; **87**: 4144-4148.
18. Hatton D, *et al.*, Two classes of cis sequences contribute to tissue-specific expression of a PAL2 promoter in transgenic tobacco. *Plant J* 1995; **7**: 859-876.
19. Verdaguer B, de Kochko A, Beachy RN, Fauquet C., Isolation and expression in transgenic tobacco and rice plants, of the cassava vein mosaic virus (CVMV) promoter. *Plant Mol Biol* 1996; **31**:1129–1139.

20. Mitra A, Choi HK, An G., Structural and functional analyses of Arabidopsis thaliana chlorophyll a/b-binding protein (cab) promoters. *Plant Mol Biol* 1989; **12**: 169-179.
21. Christensen AH, Sharrock RA, Quail PH., Maize polyubiquitin genes: Structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Mol Biol* 1992; **18**: 675–689.
22. Bovy A, *et al.,* High-flavonol tomatoes resulting from the heterologous expression of the maize transcription factor genes LC and C1. *Plant Cell* 2002; **14**: 2509-2526.