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The Impact of Gene Duplication on Genetically Modified Food Crops

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Abstract

This review has focused on Impact of Gene Duplication on Genetically Modified Food Crops. Genetically modified crops (GMC, GM crops, or biotech crops) are plants, the DNA of which has been modified using genetic engineering techniques. The aim is to introduce a new trait to the plant, which does not occur naturally in the species. Examples include resistance to certain pests, diseases, or environmental conditions, or resistance to chemical treatments (e.g. resistance to herbicides, weedicides), or the production of a certain nutrient or pharmaceutical agent. Gene duplication has a significant impact on all genomes as gene duplication contributes much of the raw material for natural selection to shape novel genes. In the context of crop evolution, interest in gene duplication has been intense as the crop genome is particularly rich in duplicated genomic regions. Genome rearrangement and speciation occurs because of genomic instability caused by gene duplication. Recent evidence suggests that crops with duplicated genes have undergone greater diversification than other crops.

Keywords: - Genetically Modified Food (GMF), Gene Duplication, *Bacillus thuringiensis* (Bt)

Introduction

In 1901, *Bacillus thuringiensis* was first discovered by a Japanese biologistShigetaneIshiwata.[1]In 1911,*B. thuringiensis* (Bt) was rediscovered in Germany by Ernst Berliner, who isolated it as the cause of a disease called *Schlaffsucht* in flour moth caterpillars. The bacterium produces a protein (Bt Protein) that is harmless until it turns toxic in the caterpillar's stomach. There an enzyme cuts the Bt protein into pieces that lock into a special receptor (protein lock) in the caterpillar's gut forming a pore and paralyzing the digestive tract. This lockingaction destroys the gut and kills the caterpillar. Adult butterflies and other insects and animals don't have any "locks" for the Bt toxin. Bt doesn't harm wildlife the way traditional pesticide sprays do. In fact, organic farmers have relied on this natural biological pesticide for years.

Scientists have incorporated the gene for the Bt protein into crops that are frequently destroyed by caterpillars. The plants produce the Bt protein in their leaves. When caterpillars eat the leaves, they die. In 1946, scientists first discovered that DNA naturally transfers between organisms.[2] It is now known that there are several natural mechanisms for flow of genes or horizontal gene transfer and

that these occur in nature on a large scale, for example, it is a major mechanism for antibiotic resistance in pathogenic bacteria.[3] This is facilitated by transposons, retro-transposons, proviruses and other mobile genetic elements that naturally translocate to new sites in a genome.[4]

They often move to new species over an evolutionary time scale and play a major role in dynamic changes to chromosomes during evolution. The introduction of foreign germplasm into crops has been achieved by traditional crop breeders by artificially overcoming fertility barriers. In 1875, a hybrid cereal was created by crossing wheat and rye.[5] Since then important traits have been introduced into wheat, including dwarfing genes and rust resistance.[6] Plant tissue culture and induction of mutations through mutagens have also enabled scientists to artificially alter the makeup of plant genomes.[7]

Methods

Genetically engineered plants are generated in a laboratory by altering their genetic makeup. This is usually done by adding one or more genes to a plant's genome using genetic engineering techniques. Most genetically modified plants are generated by the Biolistic method (Particle Gun) or by *Agrobacterium* mediated transformation. Plant scientists, backed by results of modern comprehensive profiling of crop composition, point out that crops modified using GM techniques are less likely to have unintended changes than are in conventionally bred crops.[8]

In research Tobacco (*Nicotiana tabacum*) and *Arabidopsis thaliana* are the most genetically modified plants, due to well-developed transformation methods, easy propagation and well-studied genomes.[9] They serve as model organisms for other plant species. In the biolistic method, DNA is bound to tiny particles of gold or tungsten which is then subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles penetrate both the cell wall and membranes. The DNA separates from the metal and is integrated into plant genome inside the nucleus. This method has been applied successfully for many cultivated crops, especially monocots like maize or wheat, for which transformation using *Agrobacterium* has been less successful.The major disadvantage of this procedure is that serious damage can be done to the cellular tissue.[10] Agrobacterium are natural plant parasites, and their natural ability to transfer genes provides another method for the development of genetically engineered plants. To create a suitable environment for themselves, these Agrobacterium inserttheir genes into plant hosts, resulting in a proliferation of plant cells near the soil level (crown gall). The genetic information for tumour growth is encoded

on a mobile, circular DNA fragment (plasmid). When Agrobacterium infects a plant, it transfers this T-DNA to a random site in the plant genome. When used in genetic engineering the bacterial T-DNA is removed from the bacterial plasmid and replaced with the desired foreign gene. The bacterium is just a vector, enabling transportation of foreign genes into plants. This method works especially well for dicotyledonous plants like potatoes, tomatoes, and tobacco. Agrobacterium infection is less successful in crops like wheat and maize. Introducing new genes into plants requires a promoter specific to the area where the gene is to be expressed. For instance, if we want the gene to be expressed only in rice grains and not in leaves, then an endosperm-specific promoter would be used. The codons of the gene must also be optimized for the organism due to codon usage bias.[12]

Duplication of Genes

Researchers were always inspired by the bright yellow daffodil. How did it produce beta-carotene? They found that several daffodil enzymes manufacture beta-carotene from other molecules. Rice has those other molecules, but it doesn't produce the enzymes to rearrange them into beta-carotene in its kernel. Could they give rice the genes for those enzymes and get them to work together? Previous researchers had inserted several genes that worked individually to make separate products. No one had successfully inserted a group of genes that had to work in sync to make one product. Duplication of genetic material has probably played a major role in the evolution of all genomes. Gene duplication provides the raw material for the generation of new genes, and so is one of the principal drivers of evolutionary novelty at the molecular level. They also play a role in promoting genome rearrangement and, probably, in driving speciation.

Here, I review the general role of gene duplication in genome evolution, drawing on data from a wide range of organisms. I then focus on the crop genome, describing both the pattern of duplication in the crop genome, and highlighting the particular roles gene duplication has probably played in crop evolution. The large amount of duplicated material in the crop genome suggests gene duplication may have been particularly important in crop evolution, and there is evidence that it has been crucial in the evolution of a number of uniquely crop traits during the most recent period of our evolutionary history.

A striking feature of the crop genome is the high density of segments of duplicated DNA. A total of around 13.7% of the crop genome is thought to consist of duplicated sequence. Most of this duplicated material is small, non-functional pieces of DNA that are likely to be rapidly deleted, but much of it consists of relatively large duplications that might contain intact functional elements. Pairs

of genomic regions showing over 90% sequence similarity over at least 1kb make up about 5.4% of the crop genome and most of this (5.0%) is in regions over 5kb in length. This content is certainly greater than that of most other vertebrate species, with around 2.7% of the mouse and chicken genomes and 1.6% of the rat genome being duplications over 1kb, and less than 6% being duplicated in total. Notably, the density of duplicated regions varies greatly across the human genome, varying between autosomes from 1.7% to 11.9%, and up to 50.4% for the Y chromosome.[7]

The character and density of duplicates also varies between regions of chromosomes: the presence of duplications tends to increase the local rate of origin of new duplicates, leading to hotspots with high numbers of duplicated segments. There is also variation between chromosome regions: pericentromeric regions account for about a third of duplicated material in crops, and are particularly enriched for interchromosomal duplicates, and subtelomeric regions are similarly enriched, but to a far lesser extent. Duplicated regions across the rest of the genome are mostly intrachromosomal and particularly contain clusters of tandem duplicates. These duplications also tend to be younger than interchromosomal duplications and more gene-rich.

For a duplication to persist through evolutionary time, an initially unique mutation must spread throughout a population. With two initially identical copies of a gene, one copy is probably more-or-less redundant, and so is a target for mutation, free from purifying selection. However, most mutations are deleterious, and many will disrupt the coding sequence or promoter elements of a gene, rendering it inactive or incapable and so producing a pseudo-gene. For a duplicated gene to evolve into a new function, positively-selected mutations for the new function would need to occur before any loss-of-function mutation. Such mutations seem likely to be much more common than beneficial ones, leading to a slight mystery over how exactly duplicated genes have produced the remarkable diversity of existing gene families. While most gene families must be the result of gene duplication and subsequent selection to new functions, most duplicate genes are probably quite rapidly removed from the genome.[13]

An important solution to this conundrum was the suggestion that duplicate genes could be retained through a process that, at least initially, only involves degenerative mutations. The duplications-degeneration-complementation (DDC, or sub functionalisation) model proposes that duplicate copies of genes with multiple sites of expression, or with multiple functions, become fixed when different copies lose different regulatory elements or different functional sites. This model avoids the

difficulty of requiring positively-selected gain-of-function mutations to occur before the effects of loss-of-function mutations are felt. The emphasis on regulatory elements also makes sense. As we learn more about eukaryotic gene regulation, it is becoming increasingly clear that regulatory elements may be as large, or larger, than the coding sequence for a particular gene, so these elements present a large target for mutation. A great deal of evidence supports this model: a number of gene pairs are known that have partitioned the expression pattern, splicing variants, or functions of a single-copy ancestor. Such evidence is slightly circumstantial, as such differences could have evolved following fixation by some other process – cases of exchange of expression patterns, at least, between paralogs, have been observed. In any case, theoretical models of population genetics also support the likelihood of the DDC model, and it must be seen as the likely explanation for the fixation of many, if not most, duplicate gene copies.[14]

In principle, it should be easy to establish the relative contribution of subfunctionalisation and the alternative model (neofunctionalisation): genes fixed by the latter process should show signs of positively selected substitutions, while those fixed by the former process should not. Unfortunately, things are not so easy. Following the subfunctionalisation process, there is nothing to stop positive selected changes then occurring as the genes (now free from pleiotropic constraints) adapt to more specific roles. Indeed, this might be expected to be a very frequent occurrence. It can also be difficult to detect positive selection from the ratio of synonymous to non-synonymous substitutions. There is very little statistical power to detect higher ratios when few substitutions have occurred, and choosing between the models will depend upon detecting selection acting on the very first few substitutions, which may be difficult, or even impossible.[15]

A few studies have shown that dN/dS ratios are higher among young pairs of duplicates than older duplicates, but none have shown ratios as high as 1, which is generally taken to indicate positive selection. There is thus evidence for at least a relaxation of purifying selection early in duplicate evolution, but this is probably to be expected under either of the two main models.

Conclusion

It is necessary to produce large number of GM plants to obtain one that has the desirable characters for its use as a basis of a new GM crop variety. Most of these so called conventional plant breeding methods (such as gene transfer by pollination, mutation breeding, cell selection and

induced polyploidy) have a substantially greater discard rate. Mutation breeding, for instance, involves the production of unpredictable genetic changes and thousands of undesirable plants are discarded in order to identify plants with suitable qualities for further breeding.

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