SUSTAINABLE AGRICULTURAL DEVELOPMENT: THE ROLE OF INTERNATIONAL COOPERATION

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Key Elements of Modern Biotechnology of Relevance to Agriculture

INTRODUCTION

In the present day it would be unthinkable to mount a research programme into a topic of functional biology without it having a component of recombinant DNA technology. The advances of molecular biology have led to remarkable increases in our knowledge, and in our powers to acquire new knowledge, at the cellular and molecular level of understanding of biological processes.

The integration of recombinant DNA technologies into modern biology has reached a stage where they are now influencing our agriculture. I believe it is not an overstatement to say that, as from now, it would also be unthinkable to mount a programme in agricultural research without considering the opportunity for recombinant DNA technologies to be used. This is especially true in the production of improved cultivars. Genetic engineering, one aspect of recombinant DNA technology, is poised to make significant contributions in the modification of the genetic instruction that underlies the performance of our agricultural production plants.

We now are able to consider either the addition or subtraction of particular genes in the genetic make-up of a plant. We are also able to modulate the level of expression of genes, thus influencing the amount of a particular gene product being made. In the not too distant future we will be able to consider gene replacement, so that we can upgrade the existing genetic software by replacing one version of a gene with a newer, improved version for a more desirable product. These manipulations will close the gap between yield and potential yield. They will provide plants with more robust resistance to pests and disease. They will enable the production of high-performance hybrid seed in many crop species and will allow us to construct plants whose seasonal requirements are complementary to each other in agricultural production systems.

As well as providing a more flexible and valuable entry of plant products to the food production sector, genetic engineering will enable agricultural production to have a greater impact in the pharmaceutical and industrial business sectors.

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WHAT HAS MADE BIOTECHNOLOGY POSSIBLE?

Genetic engineering is the most important way in which we will use advances in biotechnology in agriculture. Basically genetic engineering is the precise manipulation of the genetic make-up of a plant, where the manipulation involves the addition of a known gene construct. The key advance that has made this possible is the development of a series of technologies which allow us to isolate and mass-produce particular segments of DNA. We have an increasing ability now to isolate a particular gene sequence and to experiment with it. This has led to huge increases in our knowledge and understanding of gene structure and function.

Another important step has been the development of gene transfer systems. We now have methods which enable us to place new genetic material into recipient plants. Invariably this means introducing a new gene into a single cell and then recovering a whole, transgenic, plant from that single cell. Regeneration of plants from single cells, often from somatic cells, the cells from vegetative parts of plants, is an essential part of genetic engineering.

A third major factor has been the realization that a gene has two major components; a product-coding region which dictates the specific amino-acid sequence of the gene product, and a control region which determines the expression (use) of the coding segment. The control component of a gene, the promoter region, provides, to a large degree, the specificity of gene expression, controlling where the gene product will be produced in a plant, when it will be produced and in what yield it will be produced.

These three key elements of genetic engineering have given us the ability to make gene constructs, working genes with an appropriate control or promoter segment and the desired coding segment, both of which are necessary for a needed adjustment to a plant. This has opened up the doors of genetic variation to researchers involved in plant improvement. We are able to take parts of genes from many different organisms and combine them into working gene constructs. We are able to give properties to plants that are quite new; properties that previously, for example, might have been found only in bacteria or in animals.

These same advances that have allowed for the addition of novel characteristics have also provided us with the ability to modify the existing genetic material of a plant to provide a slightly changed product or to alter the expression of existing genes so as to direct a gene product to be formed in a part of a plant in which it was not previously present. The new knowledge base has also meant that we are now beginning to be able to modify the physiological responses of plants to environmental stresses and to modify the architecture of plants so they better fit the conditions of our agricultural production systems.

In general concept many of these kinds of change have been carried out by plant breeders using the technologies that have been available to them up to the present time. But breeders have been limited in what they have been able to achieve by their control of genetic variation being restricted to the sexual confines of any one species. Breeders have also been limited in synthetically producing variation by having to use random procedures to change the basic

genetic code (irradiation or chemical mutagenesis), this leading to great inefficiencies in the process of selecting required variation. The plant breeder will no longer be limited to the gene products normally found in the particular plant. If the breeder can define the need or the problem there will be a good chance that genetic engineering will provide a solution, often using gene components from other species.

WHERE ARE WE NOW?

Just what is the reality at present – will we see genetically engineered plants in our horticulture and agriculture in the near future? The answer is more dependent on legislation and societal acceptance than on limitations of our science.

Gene transfer

We are not yet able to introduce genes into every plant important in our agriculture. We are still limited, for example, in the cereal plants by not being able to introduce genes into wheat or barley. Recently, methods have been developed for rice and maize, although these methods have not yet reached a stage of general availability. With the broad-leafed plants there has been much more success. Mostly this is due to the availability of a natural gene transfer system. The soil bacterium, *Agrobacterium*, has the unique ability to introduce specific gene segments into the chromosomal DNA of its host plants. This system has been adapted for use in genetic engineering to provide a powerful gene transfer system. *Agrobacterium* acts as a gene taxi, taking our laboratory-made gene constructs into the chromosomal DNA of the target plant.

In the cereals and other monocotyledonous plants we mostly rely on physical methods for introducing specific gene segments into the host cells. Most spectacularly, gene segments can be carried into cells by high-velocity particles, often propelled by an explosive charge.

In all of these gene transfer systems the host plant provides many of the enzymes that are needed for the actual insertion of a DNA segment into chromosomal DNA. This reliance on the specificities and powers of enzymes associated with nucleic acid replication and repair has been an underlying strength in the development of recombinant DNA technologies – a genetic engineer uses natural processes to great advantage. We can confidently expect gene transfer systems for all species of plants that are important in agriculture, forestry or horticulture.

What genes can we transfer?

Coding sequences that are obviously important in plant improvement are those which dictate the production of insecticides and those which give resist-

ance to disease organisms and to herbicides. Frequently, these are coding sequences which can be taken from species other than the agricultural plant. They represent novel genetic properties. Increasingly, the new coding sequences may be synthetic, based upon naturally existing sequences or constructed by the genetic engineer on the basis of fundamental biological knowledge that has become available, largely as a result of molecular biology-based research.

An important point in regard to disease and pest resistance is that our increased understanding of interactions between organisms is enabling us to construct resistance sequences which will be longer lasting than many of the naturally occurring genes. The resistance genes can be constructed so as to present a far more difficult barrier to the pathogen or pest to overcome with its natural genetic processes. Other coding sequences that are already being used are sequences that provide for new proteins in the seed, tuber or leaf or for different fatty acids in seed oils. These changes lead to improvements in food quality, for either animals or humans. Specific modification of the nutritional properties of plant material is one aspect of genetic engineering which will be of great significance in meeting the pressures of growing world population. A genetic software system providing for a more balanced diet, with delivery through a plant seed, is likely to be one of the most user-friendly solutions to the food problem, especially in developing countries using low-technology production systems.

We will be modifying other proteins, for example the enzymes which are critical in various metabolic pathways of a plant. Again, we will be relying on increasing understanding at a cellular and molecular level of what is actually taking place in plant cell metabolism. Perhaps we will provide additional enzymes to create a branch biosynthesis pathway yielding a new product, or we may provide a modified code for a gene which will yield an enzyme with different performance characteristics from the one already existing. We can also plan to produce proteins or other molecules in parts of plants where previously they may not have been present. An example is the production of tannins in leaves of fodder plants where previously those same tannins might have been restricted to the seeds or other parts of the plant. If the addition of one or two genes could provide for this activity, it could have important consequences in the suitability of the leaf material for animal feed, avoiding problems such as bloat for the animals.

Thus far I have described the addition of coding sequences of a plant genome. It is likely that there will be as much need to subtract coding sequences. In many of our plants there are some metabolic activities that we would prefer to be there. For example, removal of an anti-nutritional factor from legume seeds (soybean) could improve the value of the seed meal in animal feeds. The technology to remove a gene product, rather than add one, has been developing rapidly in the last two or three years. We are not able in plants to do what can be done in yeast; that is, to remove a specific gene or replace a gene with a non-operative copy. But we can interrupt the information flow by causing the mRNA from the gene to be nullified in terms of informational content for protein making, or to be destroyed. If we add a gene construct which produces a mRNA containing the anti-sense strand of a gene-

coding segment, the simultaneous production of the sense and the anti-sense mRNAs leads to a molecular pairing of the strands, so that the sense mRNA is not available to direct the production of a protein product. Anti-sense technology is still in its infancy but already it has been used in modifying the genetic make-up of tomato to improve its storage and ripening qualities. The genetically engineered tomato may well be the first transgenic plant foodstuff to reach the market-place.

We also have a developing technology where a gene can be constructed to produce a mRNA containing a ribozyme which seeks and destroys a target mRNA in the nucleus. We can build a ribozyme-making gene specifically directed against an unwanted gene product. Similarly, a ribozyme-making gene can be directed against an invading virus, providing an entirely new way of combating some of the most serious diseases of agricultural and horticultural crops. In Canberra we are developing a powerful, general-purpose ribozyme technology which we call Gene Shears. It has remarkable specificity and wide applicability and is likely to be an important tool in the armoury of plant breeders of the future.

The other coding sequences that are of great importance and which will become a more common substrate in our genetic engineering activities in the future are those genes determining complex agronomic characteristics such as performance and yield. Also we will pay increasing attention to those genes controlling key stages in plant development. Most of the genes involved in yield and other multi-genically determined characters are unknown, but they are no longer beyond our reach. There are two major developments in technology which have made it possible to track genes contributing to a complex phenotype. One is the use of DNA sequence markers. Variations in nucleotide sequences are sufficiently frequent in most plants for any given DNA sequences to be likely to have a range of nucleotide substitutions surrounding it. If these substitutions create or destroy restriction enzyme recognition sites then the tag DNA sequence can be expected to be found in different length DNA segments in different genetic stocks. These DNA segment lengths are called restriction fragment length polymorphisms, RFLPs, and they can be linked to other genes by standard genetic linkage analysis.

This and related techniques of sequence tagging provide us with a powerful method to track DNA segments of major value in contributing to a complex phenotype. These segments can be tracked at a DNA level without worrying about phenotypic expression throughout a plant breeding programme. Segments with an additive or complementary effect can be brought together at a critical late state in the production of an elite genotype. These sequence markers are beginning to increase the efficiency of breeding programmes, saving generations of biological testing for many characters and permitting precise selection of wanted individuals in pedigrees. In most breeding programmes this will bring about a saving in time and resources.

Arabidopsis: a powerful new force in plant biology and agriculture

Analysis of mutants has been a valuable technique in plant breeding and genetics, but it has now become vastly more powerful, with the adoption of *Arabidopsis*, a cruciferous weed, as an experimental model plant. *Arabidopsis* has a small genome and a rapidly expanding array of molecular aids to assist in the analysis of its genome. It is also a self-pollinating plant (important in the production of mutations), capable of being cultured readily in growth cabinets under laboratory conditions, the life cycle taking only a few weeks.

These attributes have persuaded plant molecular biologists to focus on this plant and develop it as a tool for analysis and manipulation of plant genomes. It is possible to use *Arabidopsis* to obtain mutants for almost any phenotype under investigation. But beyond a genetic analysis, the traditional follow-up of mutagenesis, the other features of *Arabidopsis* make it possible for us to be confident that we can map the mutant in the genome and proceed to isolate it physically, enabling detailed analysis of the gene to be carried out.

The advent of *Arabidopsis* is leading to an acceleration of the understanding of many aspects of plant biology. It is also leading to the use of gene sequences isolated from *Arabidopsis* as probes to acquire comparable sequences from the genomes of agriculturally important plants, ones not usually so amenable as *Arabidopsis* in molecular analysis. Weed genes are leading us to crop genes!

The control segments of gene constructs

An introduced coding sequence in a transgenic plant is only as good as its system of expression control. Analyses of the control regions of genes have been of enormous importance in tackling some of the most basic concepts in plant biology. For example, we are beginning to see that cell differentiation and the processes of development in a plant's life cycle depend in large part upon differential gene expression – cell tissue-specific expression patterns.

Knowledge that control sequences immediately upstream of the product-coding region of a gene are sufficient to provide correct expression has meant that we have been able to approach agricultural problems and design solutions for them at the gene construct level. This has probably been the most important bridge enabling a meeting of these different disciplines. We already have a lot of knowledge on the types of controls that are coded into the promoter regions of many genes. We know of controls that provide for a developmental or cell-specific pattern of expression, controls that provide for expression in response to particular environmental cue or stress, and controls that modulate the level of gene expression. We know that similar control sequences in front of different genes provide for coordinate expression of the genes, despite their being distributed widely throughout the genome.

Maybe most important of all is that the majority of gene controls in plants are conserved across the plant kingdom. This is of critical importance because it has meant that gene constructs can be put together with components from a