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Fruit and vegetable biotechnology

Edited by Victoriano Valpuesta

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1

Introduction

V. Valpuesta, Universidad de Málaga

Biotechnology can be seen as an imprecise term since the harnessing of any biological process could justifiably be called biotechnology. In food processing it could reasonably be applied to processes as long established as bread making and brewing. However, the revolution in our understanding of the molecular mechanisms underlying the processes of life, in particular our understanding of DNA, has resulted in the potential to manipulate those mechanisms for our requirements. This new-found knowledge and ability is loosely termed biotechnology.

There are two main applications of biotechnology to fruit and vegetable production:

1. as an aid to conventional breeding programmes
2. its ability to transfer genes between different organisms.

Physiological or morphological traits are governed by genes carried on chromosomes. The ability to monitor the presence or absence of such genes in plants is a great aid to plant breeders. This is done through the use of molecular markers, characteristic DNA sequences or fragments that are closely linked to the gene or genes in question. Molecular biological methods allowing the monitoring of such markers in many independent individuals, for example those arising from a cross between two plant varieties. This is a great aid to the selection process.

The ability to transfer genes means that specific genes can be added to a crop variety in one step, avoiding all the back-crossing that is normally required, providing a major saving of time and effort. Furthermore, those genes that are added need not come from a species that is sexually compatible with the crop in question. Conventional breeding is, of course, limited to the introduction of

genes from plants of the same species or very near relatives. By employing the science of genetic engineering, it is possible to bring into a crop plant different genes from other plants or even bacteria, fungi or animals. Genes are, simplistically, made up of two parts: the coding region which determines what the gene product is, and the promoter, a set of instructions specifying where, when and to what degree a gene is expressed. Coding regions and promoters from different genes can be spliced together in the laboratory to provide genes with new and useful properties (recombinant DNA). These foreign or recombinant genes can then be introduced back into crop plants through the techniques of plant genetic transformation. The introduced genes integrate into the plant genome and will be passed on to the offspring in the normal way. In this way it is possible to enhance existing characteristics and introduce new attributes into a crop.

This book explores the application of biotechnology in this second area of fruit and vegetable cultivation and their subsequent use in food processing. Chapter 2 describes the basic tools and methods of genetic manipulation, from the selection and isolation of genes to safety issues such as the stability of transgenes. Part I then considers the range of target properties for genetic enhancement, starting with two chapters on how biotechnology can improve quality and productivity in fruit and vegetable cultivation. Chapter 3 looks at the genetic modification of agronomic traits in fruit crops such as herbicide resistance, resistance to plant pests and environmental stresses, increasing yield and fruit quality. Chapter 4 looks in more detail at improving plant defences against pathogens. A group of three chapters then discusses the enhancement of traits which affect final product quality. Chapter 5 considers how biotechnology can help in extending the post-harvest life of fruit and vegetables, an increasingly important issue given the complexity of modern supply chains. Chapter 6 reviews the use of molecular genetics to improve food properties such as nutritional quality and sensory characteristics such as colour and flavour. Given its importance, Chapter 7 looks in more detail at the nutritional enhancement of plant foods.

Part II includes three case studies on the application of biotechnology to particular crops. Tomato was the subject of the first commercial release of a transgenic food product, the Flavt Savr tomato with extended shelf life of the ripe fruit, and has subsequently been a particular focus for research in this field. Chapter 8 reviews the range of work. Chapter 9 considers current commercial developments with transgenic potato whilst Chapter 10 reviews work on a range of other vegetables and fruit from melon and cucumber to cabbage, broccoli, cauliflower and lettuce. Finally, Part III looks at the all-important issues of consumer attitudes and risk assessment, with chapters on these issues and identifying GMOs in foods.

2

Tools of genetic engineering in plants

J. Pozueta-Romero, Universidad Pública de Navarra

2.1 Introduction

Transfer and expression of foreign genes in plant cells, now routine practice in several laboratories around the world, has become a major tool to carry out gene expression studies and to obtain plant varieties of potential agricultural interest. The capacity to introduce and express diverse foreign genes in plants, first described for tobacco in 1984,[1] has been extended to many species. Transgenic crops such as tomato, cotton, maize, soybean, etc., are now available for human consumption and by complementing traditional methods of crop improvement (and thus becoming an integral part of agriculture), they will have a profound impact on food production, economic development and on the development of a sustainable agricultural system during the 21st century.

Although the capacity to introduce and manipulate specific gene expression in plants provides a powerful tool for fundamental research, much of the support for plant transformation research has been provided because of the generation of plants with useful and rapidly discernible phenotypes which are unachievable by conventional plant breeding, i.e., resistance to viruses, insects, herbicides, or post-harvest deterioration.[2–9] Plants useful for production of materials ranging from pharmaceuticals[10] to biodegradable plastics.[11] have been obtained using this new technology. Remarkably also, plant biotechnology techniques have been used to create plants overexpressing genes from human pathogens, the resulting plants accumulating proteins with immunogenic properties. These plants have been proved to be effective in causing oral immunization against diseases such as hepatitis B, cholera and rabies[12–14] which demonstrate the feasibility of using transgenic plants as expression and delivery systems for oral vaccines. In this chapter the technical aspects of the state of the art in plant

engineering are described. It also identifies technical problems remaining in the development of systems of plant transformation applicable to crop improvement.

2.2 Selection and isolation of genes

Genetic information is carried in the linear sequence of nucleotides in DNA. Its expression involves the translation of the linear sequence of specific regions of DNA existing in the nucleus of the cell (called coding regions or genes) into a colinear sequence of amino acids (proteins). As an intermediate step, however, DNA must be copied into a different type of polynucleotide known as ribonucleic acid (RNA) which retains all the information of the DNA sequence from which it was copied. Single-stranded RNA molecules are synthesized by a process known as DNA transcription which is regulated by interactions between DNA sequences located upstream of the gene (promoters) and proteins (transcription factors). Thousands of RNA transcripts can be made from the same DNA segment in a given cell. Many of these RNA molecules undergo major chemical changes before they leave the nucleus to serve as the messenger RNA (mRNA) molecules that direct the synthesis of proteins in the cytosol.

Fragments of DNA can be amplified by a process called DNA cloning which consists in inserting the DNA into a plasmid or a bacterial virus and then growing these in bacterial (or yeast) cells. Plasmids are small circular molecules of DNA that occur naturally in bacteria, where they replicate as independent units. As these bacteria divide, the plasmid also replicates to produce an enormous number of copies of the cloned DNA fragment. Although restricted genomic DNA fragments can be cloned to produce genomic libraries, cDNA libraries are most frequently used to isolate and characterize genes necessary for the production of genetically engineered plants. cDNA libraries represent the information encoded in the mRNA of a particular tissue or organism. mRNA molecules are exceptionally labile and difficult to amplify in their natural form. For this reason, the information encoded by the mRNA is converted into a stable DNA duplex (cDNA) via enzymatic reactions catalyzed by reverse transcriptase and DNA polymerase I, and then is inserted into a self-replicating plasmid. The resulting heterogeneous population of cDNA molecules collectively encodes virtually all of the mRNAs sysnthesized by the cell. Once the information is available in the form of a cDNA library, individual processed segments of the original genetic information can be isolated and examined with relative ease.

A representative cDNA library should contain full-length copies of the original population of mRNA. cDNA libraries provide a method by which the transcription and processing of mRNA can be examined and interpreted to produce models for the flow of information responsible for the fundamental characteristics of each organism and tissue type. Comprehensive cDNA libraries can be routinely established from small quantities of mRNA, and a variety of reliable methods are available to identify cDNA clones corresponding to extremely rare species of mRNA. As the enzymatic reactions used to synthesize

cDNA have improved, the sizes of cloned cDNAs have increased, and it is often possible to isolate cloned full-length cDNAs corresponding to large mRNAs.

Screening of recombinant clones for the search of agronomically interesting genes can be carried out effectively with only two types of reagents: antibodies and nucleic acid probes. In those instances when both types of reagents are available, nucleic acid probes are preferred because they can be used under a variety of different stringencies that minimize the chance of undesired cross-reactions. Furthermore, nucleic acid probes will detect all clones that contain cDNA sequences, whereas antibodies will react only with a subset of these clones (in some cases one in six at best) in which the cDNA has been inserted into the vector in the correct reading frame and orientation.

The higher the concentration of the sequences of interest in the starting mRNA, the easier the task of isolating relevant cDNA clones becomes. It is therefore worthwhile investing some effort to make sure that the richest source of mRNA available is being used. Whenever possible, estimates should be obtained of the frequency with which the mRNA of interest occurs in the starting preparation. mRNAs that represent less than 0.5% of the total mRNA population of the cell are classified as 'low-abundance' mRNAs. Using the protocol to generate cDNA libraries explained above, the isolation of cDNA clones from low-abundance mRNAs presents two major problems, first, construction of a cDNA library whose size is sufficient to ensure that the clone of interest has a good chance of being represented and secondly, identification and isolation of the clone(s) of interest. These problems have been overcome by the possibility of amplifying specific segments of DNA by the polymerase chain reaction (PCR) which is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that specifically hybridize to opposite strands and flank the region of interest in the target DNA.[15] Starting from minute amounts of DNA, repetitive series of cycles involving template denaturation, primer annealing, and the extension of the annealed primers by thermostable DNA polymerase results in the exponential accumulation of a specific fragment. *In vitro* amplification systems have the advantage of being specific, rapid, but above all they allow the detection and amplification of low-abundance transcripts from total RNA.[16] PCR can be also used to produce probes, DNA sequencing and *in vitro* generation of mutations in DNA molecules.

2.3 Transformation and regeneration of plants

Development of procedures in cell biology to regenerate plants from single cells and the discovery of techniques to transfer and express foreign genes to plant cells provided the prerequisite for the practical use of genetic engineering in crop improvement. The essential requirements in a gene transfer system for production of transgenic plants are the availability of a target tissue having cells competent for both plant regeneration and transformation, a method to introduce