SHORT COMMUNICATION

A COMPACT HISTORY OF GENETIC TRANSFORMATION AND ITS INFLUENCE ON CROP DEVELOPMENT

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Abstract

The term 'crop biotechnology' did not occur in the scientific literature in the 1970s and was sparse in the 1980s. In the following decade its occurrence increased by an order of magnitude, reflecting the dramatic way in which the new range of molecular genetic tools and novel approaches for investigating and manipulating living organisms influenced biological and agricultural research. Understanding and developing the process of genetic transformation was a key element in this movement, starting with studies of prokaryotic systems and advancing later to more complex ones. The trend is clearly continuing exponentially into the 21st century. Within the biotechnological revolution there have been many sub-developments producing novel biological insights, ultimately affecting crop production and strategic management of agriculture.

Keywords: transformation, genetic engineering, biotechnology, crop development, sugarcane

Early transformation discoveries

In 1928, Griffith published the now classic observation that cells of the bacterium *Diplococcus pneumoniae* could be heritably altered by association with a cell-derived 'transforming principle'. Sixteen years later this transforming substance having the potential for genetic interaction was identified as deoxyribonucleic acid (Avery *et al*., 1944). Within the next three decades the phenomenon of bacterial transformation – the uptake and integration into a recipient cell chromosome of DNA fragments released into the environment by death of a donor bacterium - had become widely identified as a natural mechanism of genetic recombination (Hotchkiss and Gabor, 1970). During the same period the phenomenon came to be exploited extensively as a means of genetic analysis, especially in the important Gram positive model bacterium *Bacillus subtilis* which lacked the conjugation and viral transduction mechanisms found in some other bacteria (Dubnau *et al*., 1967). Techniques were developed to enhance transformation. Commonly, for example, cell populations were allowed to reach a state of semi-starvation in minimal growth medium. This treatment was found to induce in the cells a state of receptivity to DNA, termed *competence* (reviewed in Mandelstam *et al*., 1982). More extreme treatments involving calcium chloride incubation at low temperature were developed that allowed previously untransformable bacterial types such as the Gram negative model bacterium *Escherichia coli* to become permeable to DNA (Mandel and Higa, 1970). *E. coli* became a lasting new genetic workhorse and variations on these transformation approaches are used today in bacterial cloning of DNA sequences (Maniatis *et al*., 1989).

From the bacterium to animal systems

As interest in bacterial transformation developed, increasing numbers of researchers turned their attention to the possibility of naked DNA mediating comparable transformations in higher organisms. Much of the early work focused on animal systems and was both ambitious and crude. It involved the injection of DNA from the tissue of one animal into a whole individual of the same species but different phenotype. The earliest published work in this area of experimentation was by a French group (Benoit *et al*., 1957) which noted significant physical alterations after treating Pekin ducks with DNA extracted from ducks of the Kaki Campbell breed. Working along the same lines, Martinovitch and co-workers (1962) obtained changes in feather colour following the injection of chicken embryos with alternative DNA administered via the venous system. Individuals of the next generation showed haemoglobin alterations as well as external colour modifications.

In general, however, attempts to achieve transformation using gross DNA treatments such as these met with a high level of failure and various papers were published to this effect. Considerably more elegant experiments were set up by researchers working with cell culture systems in which the goal was to transfer and express specific genetic markers. For example, in pioneering work meriting publication in *Nature*, Kraus (1961) demonstrated that human bone marrow cells taken from sickle cell anaemia patients, which lack the ability to produce the β^A chain of haemoglobin, may have that ability conferred upon them by incubation with DNA from bone marrow cells homozygous for normal haemoglobin.

Following the development of egg microinjection technology, the first fully regenerated transgenic mammals appeared in the 1980s and by 1988 the 'Harvard Oncomouse', genetically engineered to be cancer prone, became the first mammalian subject of a patent (Fuller, 2008). In 1989, two freeze-dried oncomouse males became the first animals to join the permanent collection at the Science Museum, London, an event which broke a rule applied since the museum's foundation, that of collecting artefacts, not organisms. These days Harvard Oncomice can be purchased from Du Pont. They cost around US$100, 100 times more than the cost of a regular white laboratory mouse.

Plant transformation

Since plants have cells surrounded by semi-rigid polymer-based walls as well as plasma membranes, transfer of DNA across their cellular boundaries was more of a challenge. By the early 1970s, plants could be cultured and regenerated from protoplasts after enzymatic cell wall removal (Takebe *et al*., 1971), a promising cellular system for transformation. Methods for direct delivery of DNA developed in parallel, including the important electroporation (Fromm *et al*., 1985) and microprojectile ('gene gun') (Klein *et al*., 1987) techniques. However, with the earlier identification of the *Agrobacterium* Ti plasmid system (Engler *et al*., 1975) and its subsequent characterisation and revelation as a potential biological agent of transformation (Schell and Van Montagu, 1977), this had become the primary focus of attention. The first fully transgenic plants were produced in the early 1980s by independent groups led by Mary-Dell Chilton and Jeff Schell respectively using tobacco and Ti plasmid delivery as the model systems (Barton *et al*., 1983; Herrera-Estrella *et al*., 1983).

Methods for direct delivery of DNA became very important for monocotyledonous plant species when it became clear that *Agrobacterium* transformation is not a universally applicable approach, but limited to certain dicotyledonous taxa. Bombardment by DNA-coated microprojectiles was an especially beneficial alternative, as it allows penetration of the

cell wall barrier and can be used with regenerable tissues such as leaf and stem explants, obviating the need for the protoplast route. Although microprojectile delivery to cells is random, and the subsequent selection of transformants in the regeneration process is labour intensive, it has become the method of choice for most graminaceous crops. Cereals are the largest contributor to dietary carbohydrate in human society, followed by sugars from sugarcane (http://www.fao.org/docrep/W8079E/w8079e0g.htm). These are the crops for which the 'gene gun' has come into prominence in genetic modification research and development. Sugarcane tissue culture and transformation technologies have been refined over a decade and a half at the South African Sugarcane Research Institute (SASRI) in the KwaZulu-Natal province (Snyman, 2004) and several proof-in-principle studies completed successfully for herbicide, *Eldana saccharina* and sugarcane mosaic virus resistance phenotypes (Snyman *et al*., 2008). Through a partnership between SASRI and the University of Stellenbosch in the Cape, metabolic manipulation enhancing sucrose accumulation in young internodes of sugarcane has also been achieved (Groenewald and Botha, 2008).

Impact of genetic engineering on biology and agriculture

Genetic engineering technologies have been major influences in two lines of development. Firstly, they have been useful as tools in unravelling the genetics of higher organisms and their biochemical and physiological functions. As a result of this, advances in fundamental understanding in many in the biological and agricultural disciplines have accelerated in an unprecedented way over the past two decades. The power of transgenesis in promoting biological progress through the study of individual genes was envisaged 20 years ago (Willmitzer, 1988) and its reality is now well documented (Table 1).

Table 1. Trends in the literature from 1960-present: publications on transgenesis and general plant/crop biotechnologies (A), and sugarcane biotechnology (B), as shown by use of keyword searches in the Biology, Life and Environmental Sciences sector of the Google Scholar database (http://scholar.google.co.za/**). Date of searches 30 April 2008.**

A

Period	Number of citations under Keywords			
	'transgenic'	**'transgenic plants'**	**'plant biotechnology'**	**'crop biotechnology'**
1960-1969	27	6	6	nil
1970-1979	79	8	3	nil
1980-1989	3 930	848	657	11
1990-1999	27 400	14 800	4 020	127
2000-2008	206 000	25 800	8 910	427

B

Period	Number of citations under Keywords	
	'sugarcane biotechnology'	**'sugarcane' + 'biotechnology'**
1960-1969	nil	3
1970-1979	nil	22
1980-1989	1	456
1990-1999	18	1 710
2000-2008	43	5 470

Secondly, these technologies have been the means of producing commercially useful novel genotypes, in some cases for factory type production (pharmaceuticals from the milk of sheep such as 'Tracy' and 'Dolly', for example) (Campbell *et al*., 1996), in other cases for primary production in horticulture and agriculture (genetically modified crops). Since 1996, farming of approved GM crops has showed a sustained double-digit percent annual growth rate and now constitutes 114.3 million hectares of production in 23 countries, over half of which are considered to have 'developing' economies (James, 2007). Table 2 shows the predominance of the USA, Argentina and Brazil in this trend, and the position of South Africa as a significant producer of GM cultivars of maize, soybean and cotton.

Projections indicate that sugarcane may join the ranks of listed commercial GM crops as soon as 2010 if current Brazilian field trials are successful. Three varieties have been modified to exhibit sugar levels 15% higher than those of non-GM genotypes. It has been calculated that, by applying transformation technologies, the ethanol yield of sugarcane can be doubled from 6 000 litres/ha to more than 12 000 litres/ha within the next 15 years (The Financial Express, posted online 31 March 2008).

Table 2. Global area of biotech crops in 2007, ranked by country.
(Source: Clive James, 2007)

Rank	Country	Area (million ha)	Biotech (GM) crops
1*	USA*	57.7	Soybean, maize, cotton, canola, squash, papaya, alfalfa
2*	Argentina*	19.1	Soybean, maize, cotton
3*	Brazil*	15.0	Soybean, cotton
4*	Canada*	7.0	Canola, maize, soybean
5*	India*	6.2	Cotton
6*	China*	3.8	Cotton, tomato, poplar, petunia, papaya, sweet pepper
7*	Paraguay*	2.6	Soybean
8*	South Africa*	1.8	Maize, soybean, cotton
9*	Uruguay*	0.5	Soybean, maize
10*	Philippines*	0.3	Maize
11*	Australia*	0.1	Cotton
12*	Spain*	0.1	Maize
13*	Mexico*	0.1	Cotton, soybean
14	Colombia	<0.1	Cotton, carnation
15	Chile	<0.1	Maize, soybean, canola
16	France	<0.1	Maize
17	Honduras	<0.1	Maize
18	Czech Republic	<0.1	Maize
19	Portugal	<0.1	Maize
20	Germany	<0.1	Maize
21	Slovakia	<0.1	Maize
22	Romania	<0.1	Maize
23	Poland	<0.1	Maize

*There are 13 biotech 'Mega-Countries' growing 100 000 hectares or more of GM crops. South Africa is one of these, at 1.8 million hectares.

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