The World Biotechnology Situation

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Biotechnology is defined as the application of organisms, parts of organisms or sub-cellular entities, or biological processes, to manufacturing and service industries. These industries include agriculture, horticulture, forestry, the food and pharmaceutical industries, and environmental management. The aims of biotechnology encompass food and biomass production, the generation and propagation of new types of organisms, production of chemicals and useful products, exploitation of fermentation processes, diagnosis and treatment of diseases, and the identification of biological intellectual property. Recent technological and intellectual advances in molecular genetics have, within a decade or so, given rise to a highly pervasive, multidisciplinary subject - the new biotechnology. Conventionally, technology is usually defined as applied science of commercial value; biotechnology by way of contrast is employed widely to study the basic processes of life and thereby increase fundamental scientific knowledge.

• Higher Plants

Higher plants share several characteristics as life forms, distinguishing them from all the other major groups of living organisms and sub-cellular organisms (Table 1). Nonetheless, with the development of techniques to overcome barriers to sexual reproduction, as well as various methods to insert genes or sequences in the DNA of receptor organisms and sub-cellular entities containing nucleic acid, the inherent similarity of the genetic language in the major groups of organisms can be demonstrated and exploited.

Table 1. GENERAL CHARACTERISTICS OF PLANTS

1. Eukaryotic organisms based on a cellular construction. More advanced

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forms show alternation of generations.

2. Autotrophic mode of nutrition arising from the possession of intracellular chloroplasts that are responsible for photosynthesis.
3. Complex carbon metabolism giving rise to special type of squalene cyclisation and production of elaborate cell walls. Considerable cellular heterogeneity. Living cells exhibit turgor.
4. Lack of cell and organism motility; cells often joined by plasmodesmata.
5. Open-ended development of modular growth habit by virtue of retaining apical and lateral meristems.
6. Growth patterns subject to influences of the environment with specialised detection systems and biological clocks. Light (spectral composition, intensity, temporal distribution/photoperiod), temperature (day/night and root/shoot differential requirements as well as various chilling and high-temperature requirements), gravity, wind-speed, humidity, rainfall, edaphic factors, allelopathic and allelomediatory effects, pests, diseases and grazing all affect growth patterns giving rise to considerable design plasticity.
7. Unique positional signalling systems; no nervous system.
8. Single parenchymatous cells able to generate whole plants.
9. Reproductive strategies can be sexual and/or asexual, and may involve a juvenility period. Dormant dispersal units or structures may be produced.
10. Complex mass transport pathways: the xylem is dead when functional for transport of water and mineral salts; the phloem transports the products of photosynthesis and nitrogen metabolism. Transport of solutes and growth factors may be apoplastic or symplastic.
11. Cellular and physiological differentiation associated with vacuolation and susceptibility to senescence processes. No immune system.

The potato is a crop species derived from relatively few of the 228 wild, tuber-bearing taxonomic species of the genus *Solanum*, natives of the Peruvian-Bolivian Andes. Potato plants are characterised by the asexual production of tubers which are the swollen tips of diagravitropically growing modified stems - the stolons. Tubers of different types of potato vary in their size, number per plant, colour, flavour, chemical composition, toxic principles, dormancy of the spirally arranged buds ("eyes"), and tuber production is strongly influenced by light intensity (radiant flux density), light quality (spectral composition), water-use efficiency, nutrient-use efficiency, plant spacing, temperature, pests and diseases. The crop can be propagated sexually through true botanical seed, or asexually through tubers and/or tissue culture. Comparatively speaking, the potato can be relatively

easily propagated through tissue culture, but certain types are more difficult than others. Although capable of being manipulated genetically both by conventional breeding methods and modern biotechnology, it has a complex genome. Most commercial types are tetraploids although some cultivated potatoes are diploid (*eg S. phureja*). Should distinguish diploid *spp.* (often native-grown) from tuberosum. As the fourth most important crop worldwide, potato tubers are noted for their large, readily extracted starch grains, nutritious value and cookability.

In experimental terms, the potato plant is studied as a model system for sink (tuber) - source (rest of plant) relationships, starch and protein biosynthesis, pest and disease resistance, and resistance to abiotic stress. Such advantages are offset somewhat by its complex genome and relative scarcity of mutants, compared with other model species such as *Arabidopsis*.

- **Plant Breeding**

Central to the ability of the bulk of the population to move from full-time, subsistence-level food cultivation and harvesting to engage in social and technological advancement is the provision of improved crop plants. Plant breeders have always been involved in genetic engineering. Characters including yield performance, resistance or tolerance to pests and diseases, quality components, uniformity and lack of prolonged dormancy periods represent the main selection criteria. Together with advances in automation, storage and processing there is a widely held view that plant breeding will perpetually answer basic nutritional needs for burgeoning populations. This is unacceptably complacent unless the levels of investment in breeding and its associated science are sustained.

Plant breeding programmes are protracted, expensive and are rarely allowed to proceed over a period of years without interference. Basic to the needs of such programmes is access to genetic resources for parental material. Unfortunately, there has been severe attrition of genetic diversity by losses of diverse wild habitats, traditional farming area, valuable collections and obsolete landraces. Breeders need to screen vast numbers of clones over many years, carry out regional trials, multiply stocks, access statutory trials and be involved in marketing. Other problems faced include imprecise predictions of genotype by environmental interactions, incompatibility systems between and within species affecting the ability to cross-breed, juvenility or ripeness-to-flower phases,

seasonal growth patterns, changing disease virulence patterns and disease vector distributions, and complex breeding objectives involving polygenic characters. Conventional plant breeding is well-established but needs to be supplemented by bioengineering technology to allow the identification of and access to new sources of genetic variability, to speed up the process, to unravel the complexities of genomes (genetic constitutions), to understand the processes involved in breeding, and to improve the prediction of performance of products arising from the breeding programmes. Supplementary techniques such as limited chromosome transfer, somatic hybridisation, linked molecular markers *etc* are now becoming breeding tools.

Plant breeders and geneticists, physiologists, biotechnologists, pathologists, agronomists and engineers, using advanced mathematics, chemistry and physics are providing new, improved, low-input cultivars with extended growing seasons, as well as better cultivation, protection, harvesting and storage technologies. This R&D combines fundamental, strategic and applied research approaches, usually within a single project. Natural tolerance or resistance to biotic and abiotic stress whilst sustaining quality, yield performance and efficiency of production are foremost in the minds of major companies and institutions, but to the intellectual challenges are added products for the rapidly expanding biotechnology, food processing and environment industries.

- **Engineering Plants**

Modern technologies are reducing the reliance on a combination of serendipity and bulk selections for plant breeding and food processing qualities.

Aided by the rapid uptake of biotechnology in the higher education sector, and relatively crude but accurate assessments of its potential by decision-markers in government and private industry, genetic engineering of crop plants is a world-wide phenomenon, but at this juncture based mainly in the USA. Selective herbicide resistance to aid better crop management; introduction of plant-derived insecticidal genes (e.g. protease inhibitors); introduction of characteristics associated with resistance to pests, diseases, abiotic and biotic stresses; enhanced quality (e.g. amino acid composition); production of engineered oils, proteins, carbohydrates, enzymes etc. are examples of projects currently underway using several crops or related species.

The diagnosis and quantification of disease organisms are increasingly reliant on biotechnology, as are studies on the relationships between different races, pathotypes and virulence groups. To investigate the mode of action and effectiveness of control agents requires the new technologies, *eg* recombinant antibodies.

Plant breeding is thus one of the leading beneficiaries of genetic engineering. All parts of normal breeding schedules are being revolutionised, from describing the genetic architecture of parental material, overcoming natural breeding barriers and selections, to propagation, prediction of performance and identifying more accurately the added-value properties of the progeny. Speed is of the essence, so is protection of intellectual property.

New plant varieties arising from traditional breeding methods are protected in many countries by plant variety (or breeder's) rights (PVR), without recourse to patent law. Patents are now being granted for the protection of recombinant methods for the production of transgenic plants and their resultant products. Attempts are being made to harmonise patent law and practice internationally. Ethical concerns are expressed about patenting life-forms and claiming ownership. There is freedom to research under both patent and PVR law, but freedom to commercialise is complex, and therefore plant breeders using modern technologies seek protection of both types of law.

Patent protection is unlikely to affect access to existing germplasm and traditional varieties. Genetic resources and diversity are internationally seen as the common heritage of humankind. Biotechnology should and will add to genetic diversity. Counter-arguments centre on the farmer's privilege to save seed to produce subsequent crops without royalty payments to recoup R&D costs, abuse of monopoly provisions, "ordre public" and the public interest, and also the nature of more discovery. Unfortunately for all concerned, a patent of invention does not guarantee a reward for the inventor; simply put, it gives an opportunity for the inventor or patent proprietor to profit from the invention where there is a profitable market for it. Secrecy in some cases is the best commercial protectant in the short term.

Major crops excluding potato that have been successfully transformed genetically are shown in Table 2.

Table 2. GENETICALLY ENGINEERED MAJOR CROP PLANTS, EXCLUDING POTATO

Species	Transformation method	Field Trials
Banana	Bombardment/*Agrobacterium*	
Barley	Bombardment	Virus resistance
Bean	Bombardment	
Canola	Bombardment/*Agrobacterium*	Herbicide tolerance; pollination control
Cassava	Bombardment/*Agrobacterium*	
Maize	Bombardment/*Agrobacterium*	Insect resistance; herbicide tolerance
Cotton	Bombardment/*Agrobacterium*	Insect resistance; herbicide tolerance
Papaya	Bombardment/*Agrobacterium*	Virus resistance
Peanut	Bombardment/*Agrobacterium*	Virus resistance?
Poplar	Bombardment/*Agrobacterium*	Herbicide tolerance
Rice	Bombardment/*Agrobacterium*	Herbicide tolerance
Soybean	Bombardment/*Agrobacterium*	Herbicide tolerance
Squash	Bombardment/*Agrobacterium*	Virus resistance
Sugarbeet	*Agrobacterium*	Herbicide tolerance, virus resistance and anti-bolting
Sugarcane	Bombardment	
Sunflower	Bombardment	
Tomato	*Agrobacterium*	Delayed ripening; virus resistance
Wheat	Bombardment	

From P. Christou. Transformation Technology Trends in Plant Science. 1, 423-431, 1996.

In crops such as soybean, rice and maize, gene-transfer methods have been developed that do not appear to be restricted by cultivar or genotype. Nonetheless, compared with the wide range of existing crop species, few crops have received detailed attention. Moreover, most key traits are multigenic, raising more challenging questions over gene interactions and expression patterns.

For transgenic plants to achieve a small portion of their potential, basic studies will need to expand on the factors (e.g. gene promoters etc.) regulating the expression

of introduced genes in different organs and tissues at various phases of growth and differentiation. Industry would be assisted by studies on the biosynthesis and degradation of complex natural polymers such as lignin, cutin, suberin and cellulose, manifestations of cell differentiation, and the cellular components of industrial relevance. Single-gene studies will give way to polygenic-linked constructions.

- **Potato**

The relative importance of potato as a target crop species for improvement by genetic manipulation was indicated by a recent survey by Biobridge Publications on biotechnology in the European Union. Within the 1899 projects examined in survey crops the most represented, in descending order, were potato, oilseed rape, wheat, maize and barley. This was despite the fact that maize, sugarbeet and sunflower were identified as the most profitable crops for the seed industry. Another sobering observation was that the effort put into projects involving potato and oilseed rape outweighed the value of the crop. However, one must consider the fact that a significant investment in basic research is required before gene expression can be regulated in a tissue- and time-specific manner and with high efficiencies of recovery of transgenic lines with "trueness-to-type". One must also be in a position to select the most relevant homologous or heterologous genes to exploit commercially, which again requires substantial inputs of resources in the initial stages.

Efficiency of transformation and phenotypic variation in transgenic plants

The basic premise of exploiting genetic manipulation is that the ensuing material will have improved properties compared with the parents and that the plants which are regenerated maintain the key characteristics of the original cultivar. Belknap *et al.* (1995), using Lemhi Russett and Russett Burbank transformed with constructs containing GUS, ClaSP (tyrosine-rich arylphorin or a gene encoding a bacterial lytic peptide), showed that the highest potential for deviation from typical performance occurred in yield and tuber-size gradings. The frequency of off-types varied from 15 to 80% depending on cultivar, but off-types were not always apparent until grown in the field. In the case of lines transformed with GUS, less than 50% were able to produce seed tubers under field conditions. The data confirmed that the incidence of off-types is cultivar-dependent (presumably inferring variability in somaclonal variation), that glasshouse trials are no substitute for extensive field trials (at least for commercial attributes) and that

transformation can be highly unpredictable with regard to gene integration and expression. Dale and Hampson (1995) also followed the transformation efficiency of 34 potato cultivars using the tuber disc protocol used extensively for cv. Desiree. Only half of the cultivars regenerated. From those that could be regenerated all but one produced transgenic plants. Some cultivars which would not regenerate from tuber discs did so from leaf and internode segments. Some cultivars could not be transformed with either method. In Europe, the cultivar Desiree is commonly used for basic research on transgene expression. Commercial scale transformations to yield true to-type-plants with the desirable level of transgene expression and trait modification may clearly provide a more onerous task if relatively recalcitrant cultivars are to be exploited.

Pest and Disease Resistance in the Potato

- **Viruses**

Resistance derived from transformation with viral genes

Potato was one of the first crop plants to be targeted for genetic engineering for virus resistance because of the relative ease with which potato can be transformed, and because potato is particularly prone to the build-up of viral diseases during vegetative propagation. Furthermore, the newly-introduced transgene need only be present in a heterozygous state in potato, whereas in crops propagated from true botanical seed, the transgene must be present in a homozygous state to avoid segregation and loss of the transgene in a proportion of the progeny seedlings. Genetic engineering offers the greatest opportunities when used to develop resistance to viruses for which there are no natural host genes, or where host genes may be only partly effective or under multigenic control and difficult to manipulate in a breeding programme, *eg* potato leafroll virus (PLRV) and potato mop-top virus (PMTV). One of the best documented approaches is coat-protein (CP) mediated resistance, now shown to be effective against many potato viruses including PLRV and PMTV as well as potato virus X, potato virus Y. Other approaches involve the use of sequences encoding viral RNA-dependent polymerases or 'replicases', viral movement proteins and viral proteases involved in processing polyprotein gene products. Sequences have been expressed in translatable and non-translatable forms, and in sense as well as antisense orientations. In some cases, translation of protein is an essential requirement of the resistance, in others resistance is mediated by the presence of RNA transcript

and the phenomenon of co-suppression has been described as an underlying mechanism of the resistance expressed. Much more work is needed before the mechanisms of transgenic resistance are fully understood.

The situation with transgenic resistance to PLRV is particularly noteworthy because it is the most destructive virus of potato world-wide. Furthermore, although sources of host resistance are available, these are only partially effective and are difficult to manipulate in a breeding programme. Several laboratories and commercial companies have been working to develop effective CP-mediated resistance to PLRV and some success has been obtained. There have been extensive field trials in USA and the prospects are promising for early commercialisation, although there is further work to be undertaken before this form of resistance can be used throughout the world with the assurance of success. One interesting approach is that taken by Barker *et al.* (1994) in which potato clones with strong host-mediated resistance to PLRV were transformed with the PLRV CP gene. The effect of the host resistance and that provided by the CP transgene was additive and in the best transgenic lines, resistance to PLRV accumulation was as great as that found in the non-tuber-bearing *Solanum brevidens* which is the most resistant wild potato species known. This combinatorial approach is likely to be used a great deal in future in order to complement the effect of host genes and transgenes, and to combine transgenes mediating different aspects of virus resistance.

Plant-derived genes

Lodge *et al.* (1993) successfully engineered potato to express high levels of pokeweed antiviral protein (PAV) which had conferred resistance to mechanically inoculated PVX and PVY. PAV is an example of a gene encoding antiviral proteins/polypeptides also known as ribosome-inactivating proteins (RIPs) which can modify ribosomal RNA and interfere with translation. Gaffney *et al.* (1993) engineered plants to degrade endogenous salicylic acid - a compound which, when applied to plants, induces a subset of Pathogenesis-Related (PR) proteins which are normally induced following pathogen recognition. The transgenic plants were unable to establish systemic acquired resistance (SAR), adding weight to the argument that induced expression of PR genes forms an integral part of the SAR response.

Non-pathogen-derived transgenes

A limitation with pathogen-derived resistance is that it generally only acts against closely related viruses. It would be valuable to obtain a type of non-specific viral resistance. One candidate sequence is the gene for mammalian 2'-5' oligo-adenylate synthetase. This gene is activated by dsRNA and it, in turn, activates a latent endoribonuclease (RNaseL) which degrades viral RNAs. Transgenic potato plants expressing the murine 2'-5' adenylate synthetase gene have been shown to be resistant to PVX infection (Truve *et al.* 1993), and Mitra *et al.* (1996) have shown that a similar system is capable of inducing broad-spectrum resistance to tobacco mosaic virus, alfalfa mosaic virus and tobacco etch virus.

An alternative approach is to express antibody-like molecules in plants by transforming them with cDNA encoding a single chain fusion protein containing parts of the H and L chains of a Mab. The *in planta* expressed scFv antibodies provide a type of immunization which has been shown to be effective in modulating target molecules to which the antibody reacts (Owen *et al.* 1992). Tavladoraki *et al.* (1993) have shown that transgenic plants expressing antibodies that react with the coat protein of artichoke mottled crinkle virus, were more resistant to infection by AMCV than were control plants. Whether or not these alternative approaches to the use of pathogen-derived sequences can be developed into a practical form of resistance, remains to be seen. Nevertheless, these examples illustrate the ingenuity which is being deployed to develop novel forms of genetically engineered resistance.

Natural host resistance genes

Currently there are major efforts to clone the Rx and Ry genes using map-based cloning strategies. These genes confer extreme resistance or immunity to PVX and PVY, respectively. One short term objective would be to obtain linked molecular markers to these genes which could be used to develop rapid molecular screening techniques for resistance. Longer-term objectives are to use cloned genes for transformation of particular target potato cvs lacking virus resistance, then other crop species where effective host genes are not available. Another objective is to broaden the specificity of elicitation of the resistance response to obtain broad spectrum resistance which cannot be overcome by resistance breaking isolates.

- **Bacteria and Fungi**

Some of the most successful strategies have come from the use of genes coding for lytic enzymes derived from bacteria or insects. For example, During *et al* (1993) directed the expression of a bacteriophage T4 lysozyme to potato cell walls and reduced the extent of tissue maceration when plants were challenged with *Erwinia caratovora.* Destefano-Beltran *et al.* (1994) described the use of genes encoding cecropins, a potent group of antibacterial factors found in the cecropia hemolymph. They also provide a listing of putative "disease resistance" gene constructs utilising lytic peptides. The cecropin peptides, which are non-phytotoxic, possess a broad spectrum of antibacterial activity against both gram positive and gram negative forms and represent a class of lytic peptides which include maganins, attacin and melittin. Watanabe *et al.* (1995), using *Pseudomonas solanacearum* as the test organism described delayed symptoms, disease severity and mortality for plants expressing a synthetic Cecropin B gene (*Shiva -1*) which showed excellent antibacterial activity. In a recent paper, Montanelli *et al* (1995) analysed resistance in transgenic Desiree expressing the cecropin gene following a challenge with a virulent strain of *P. solanacearum.* Immunity was not observed but a group of transgenics did show resistance levels and disease development curves comparable to the field -resistant cv Cruza 148 (supplied by CIP).

Wu *et al.* (1995) used an alternative approach, exploiting the fact that plant defence responses to pathogens involve the production of active oxygen species including hydrogen peroxide (H_2O_2). They showed that growth of *E. caratovora* and *P. infestans* in culture was reduced by including *A. niger* glucose oxidase in the medium (glucose oxidase converts glucose to gluconic acid and H_2O_2. Similarly, growth in culture was severely inhibited by 100 μM H_2O_2. Transgenic potatoes expressing an *Aspergillus niger* glucose oxidase gene showed improved resistance.

Yao *et al.* 1995 have modified flux through the shikimate and phenylpropanoid pathways by introducing a tryptophan decarboxylase gene from *Catharanthus roseus* into potato tubers. The plants accumulate tryptamine rather than tryptophan and phenylalanine and , due to pathway modification, accumulate much less chlorogenic acid than the wildtype controls following wounding. Furthermore, treatment of transgenic tuber discs with arachidonic acid, an elicitor of the defence response, did not induce the normal increase in soluble and cell-wall phenolics. The transgenic tubers were more susceptible to inoculation with zoospores of *Phytophthora infestans.* This clearly shows that phenols are

involved in the plant 's defence strategy. Zhu and Chen (1996) demonstrated that potato plants expressing high levels of the plant defence gene "osmotin" increase resistance to *P. infestans,* particularly at the early phase of infection.

- **Nematodes and Insects**

Research is focused on the search for promoters to drive expression at feeding sites only and a few have been reported. Otherwise, emphasis is placed on the expression of specific lectin genes, e.g. Concanavalin A, to bind nematode sensory apparatus and inhibit feeding. Approaches involving expression of cytotoxic genes and enzyme inhibitors (similar to those described above for bacteria and fungi) are also in vogue. At present, information on the outcome of these approaches is scarce, but results on glasshouse and field testing of transgenics are expected in the U.K. and elsewhere in Europe within the next one to three years. The expression of genes encoding the *Bacillus thuringesis* toxin is now well known to protect against insects such as tuber moth and Colorado beetle but the narrow specificity of individual Bt toxins probably excludes any cross protection against nematodes. However, there are some patent claims that some Bt toxins are active against nematodes, flatworms and protozoa.

More than 50 genes encoding Bt toxins have been characterised and the toxicity of corresponding proteins is high (usually specific to orders of insect such as Lepidoptera, Diptera, Coleoptera). The degree of toxicity depends upon proteolysis in the insect gut to produce active fragments. The most successful Bt genes are those which are synthetic, highly refined DNA structures, with low sequence homologies with native bacterial genes. Fear of accelerated insect resistance development has caused some to argue that transgenic resistance should form part of an integrated pest management strategy (Tabashnik, 1994).

Stress Physiology

Potatoes are grown in a range of climates throughout the world. Even within a relatively localised geographical area, seasonal extremes in weather patterns will modify crop performance, affecting efficiency of production, yield and quality. Whilst resistance to (or tolerance of) drought, salt high and low temperature stresses are likely to examples of polygenic traits, evidence is accumulating that inroads can be made through the application of transgenic biology.

a) *Cold stress*

Cold-hardy plants produce antifreeze proteins which are effective at preventing ice growth and re-crystallisation at concentrations several orders of magnitude lower than colligative compounds. Guerra *et al.* (personal communication) have expressed genes for Type 1 antifreeze proteins (AFP's; also common acronym for Anti-Fungal Proteins) from winter flounder (*Pseudopleuronectes americanus*) in potato. Such genes are found in a range of over-wintering organisms including teleost fish, fungi and vascular plants. The protein expressed by Guerra *et al.* is alanine -rich and amphiphilic. The Type 1 AFP adsorbs to the crystal lattice of ice by hydrogen bonding *via* exactly aligned threonine residues, creating a barrier to the addition of water molecules and slowing the rate of ice crystal growth. The gene used by Guerra *et al.* was synthesised *de novo* and designed to improve codon usage in plants. Transgenic Russett Burbank plants carrying a phytohemogluttin -AFP construct showed a substantial decrease in electrolye leakage from leaves following a pre-treatment at -2^{o} C for 2 hours. The response of transgenics was similar to that of cultivar Ch'aska, a frost-resistant Bolivian genotype.

b) *Photo-oxidative stress*

Perl *et al.* (1993) expressed, in potato, genes encoding cytosolic and chloroplastidic forms of Cu- and Zn-dependent superoxide dismutases (SODs) from tomato. SODs are enzymatic free-radical scavengers, believed to protect cells from the ravages of specific active oxygen species. *In vitro* generated potato shoots were infiltrated with paraquat to generate superoxide radicals and assessed for changes in the relative quantum yield of photosynthetic oxygen evolution. Several independent transgenic lines containing either chloroplastidic or cytosolic forms of SOD showed paraquat tolerance. The approach offers a route to protection against photo-oxidative damage and, as yet , undefined stresses.

c)*Desiccation and mechanical wounding*

Jasmonic acid (JA), which is synthesised from linolenic acid via oxidation pathways, is believed to act a signal molecule in transduction pathways leading to expression of plant defence genes following pathogen attack. JA has also been ascribed a role in several physiological processes including responses to desiccation and mechanical wounding. Harms *et al.*(1995) expressed a flax allene oxidase gene in potato . The allene oxidase protein is a member of the cytochrome P450 family of hemoproteins, implicating the oxidase in the JA biosynthetic pathway. Under the control of the constitutive 35S CaMV promoter, JA levels in

leaves were increased up to 12-fold (to concentrations found in wounded wildtype plants). Mechanical wounding or desiccation elevated the concentration of JA in transgenic lines even further. However, the expression of the wound-inducible *pin 2* gene was not elevated in non-wounded transgenics. It appears that JA alone does not trigger constitutive expression of wound or water stress inducible genes. JA did not influence leaf water status, despite other reports which indicate decreased photosynthesis performance and increased stomatal conductance in response to JA application.

Developmental Biology

a)*The role of photoreceptors*

Phytochrome is a photoreceptor for red (R) and far red (FR) light and which, through the sensing of R:FR ratios, regulates key developmental processes including flowering, seed germination and the normal development of leafy shoots. Five phytochrome gene have been isolated from *Arabidopsis* but the best characterised are PHY A and PHY B . PHY A accumulates in the dark but is rapidly depleted upon conversion to the labile Pfr sensitive form. PHY B is more light-stable and is expressed at low but constitutive levels in light- and dark-grown plants. The phyochrome A (*PHY* A) gene has been constitutively up- and down-regulated in potato by Heyer *et al.*(1995). Modified expression of PHY A affected hook opening in etiolated sprouts transferred to continuous FR light. With antisense plants, hook development was suppressed, whereas transgenics constitutively over-expressing the gene showed earlier hook opening and accelerated leaf expansion at both the apex and sub-apical nodes. In wildtype controls, sprout growth was inhibited by R (less so by FR). With PHY A constitutive over-expressers, FR is as effective as R in inhibiting sprout growth. These data represented first-stage analysis of the transgenic plants but clearly showed that photomorphogenic responses can be modified.

b) *Flowering*

Transformation with an antisense sequence of a mitochondrial citrate synthase gene, delayed flower bud formation in potato and caused flower abortion once buds did form (Landschutze *et al.* 1995). Citrate synthase catalyses the first reaction in the tricarboxylic acid cycle. The reduced capacity for ATP formation may be associated with cytoplasmic male sterility. Surprisingly, vegetative

growth was not affected.

c) *Tuberisation, polyamines and ethene (ethylene)*

A gene encoding S-adenosylmethionine decarboxylase (SAMDC) has been isolated from tuberising stolon tips of potato (Kumar *et al.* 1996). SAMDC is a key enzyme in the biosynthesis of the polyamines spermine and spermidine. Polyamines are ubiquitous in all living organisms and appear to be essential for normal growth and development. They are generally abundant in actively dividing young tissues and non-senescing organs. However, their physiological roles are unclear. Potato plants containing antisense SAMDC under the control of the 35S CaMV promoter showed a range of stunted phenotypes with highly branched stems, short internodes, small leaves and inhibited root growth. This not only correlated with reduced polyamine content but also with elevated rates of ethene (ethylene) evolution (up to 50-fold). This relates to the fact that SAM, the substrate for SAMDC, is also a precursor in the ethylene biosynthesis pathway. Inhibiting polyamine formation leads to a boost in ethylene production. It is not certain yet whether the transgenic phenotype observed is due principally to reduced polyamine content of increased ethylene production. More recently, SAMDC has been antisensed at the SCRI using the tuber-specific promoter patatin. Tuberisation of cv Desiree *in vitro* is inhibited (stolon growth continues). First indications are that over-expressing lines produce a higher incidence of sessile tubers.

Molecular farming, alternative uses and added-value

Significant interest has developed in the production and storage of novel compounds, (including pharmaceuticals) and metabolites in transgenic plants. Plants, as a renewable energy source, may offer an attractive alternative to production in fermenters, for example, but only if product yields and efficiency/ease of extraction are economically viable.

a) *Pharmaceutical/medical*
Pen *et al.* (1993) have produced a number of modified forms of Human Serum Albumen in both potato and tobacco, with yield of up to 0.2g per Kg of total protein in all plant parts. This may be a viable approach since protein is yielded in the liquid fraction following potato starch extraction. Similarly, Goddijn and Pen (1995) have expressed in potato a gene encoding a heat-labile enterotoxin

(LT-B) subunit (from *E. coli*) fused to a microsomal retention signal. When fed to mice at very low concentration the plants provided successful immunisation against hepatitis B. This opens up a potential route to the production of edible vaccines. Pen *et al.*(1993) have described transgenic tobacco capable of accumulating metabolites which can induce the production of anti-toxin antibodies. Similarly, gene sequences encoding recombinant antigen binding fragments of antibodies (or "plantibodies") can be stably expressed in tobacco.

The transgenic approach has permitted the production, in plants, of several classes of foreign proteins with medicinal applications including immunogens for vaccine production, hormones, cytokines and even whole antibodies. However, levels of foreign protein expression are fairly low and usually range from 0.01-1.00% of the total soluble protein (TSP) in a plant. TSP itself is only about 4-5% of the dry weight of a plant which comprises mostly fibrous, structural, storage or soluble carbohydrates. Up to 90% of the wet weight of a plant is, of course, water.

Gene expression vectors based on plant viruses offer several advantages over transgenic plants for foreign protein production. First, plant viruses can now be genetically modified easily and rapidly, and plants infected with the modified virus can be obtained within a few weeks, rather than the months (or even years) required to produce a transgenic plant line. Second, a foreign gene contained in a plant viral genome may be expressed at far higher levels ($\geq$100x more) during the natural infection process than could ever be achieved from a nuclear DNA transgene. Third, the number of plants which produce the foreign protein can be increased simply by extracting leaf sap containing the modified virus from one infected plant and manually or mechanically inoculating it onto many healthy seedlings. Fourth, plant virus vectors can be used to express foreign proteins in plant species for which no stable DNA transformation or plant regeneration protocols have yet been developed.

One particular application of plant virus vectors, which has received considerable media attention, is to produce vaccines. In this application, a foreign nucleic acid sequence encoding a short antigenic peptide derived from a mammalian pathogen is inserted into, or fused to, the CP gene of the virus. The foreign antigen is then displayed on the surface of virus particles propagated in plants. In effect, each plant virus particle acts as a multivalent, macromolecular "carrier" to amplify, stabilize and enhance the immunogenicity of the peptide. This epitope or subunit approach to vaccine production is an attractive alternative to present methods

involving animal or human cell cultures and live, attenuated or killed, whole pathogenic agents. Plant viruses have no intrinsic pathogenic effects on humans or animals. Thus, the use of a clinically benign plant virus vector to provide a peptide vaccine avoids the use of any intact human or animal pathogen, removing the risks this entails. Furthermore, unlike microbiological or mammalian cell culture, blood products etc, an edible crop plant, used to grow a plant virus vaccine vector, is extremely unlikely to be contaminated with extraneous human or animal pathogens.

By serendipity, Michael Wilson and colleagues at SCRI have used another plant virus, potato virus X (PVX), to develop a revolutionary platform vector for high-level expression of large foreign proteins in plants. Initially, it was intended to make only freely soluble proteins, released from the PVX CP by a novel cleavage strategy; however, early and completely counter-intuitive results revealed that this virus had the capacity to assemble CP subunits fused to very large, so-called OVERCOAT™, protein moieties.

PVX, like TMV, forms cylindrical virus particles in which the genomic RNA is encapsidated in a helical array of identical CP subunits (about 1300 in PVX), but PVX virions are slightly flexuous. No X-ray-derived structural information exists for PVX, but other lines of evidence indicate that the N-terminus of the CP is exposed on the outer surface of virus particles.

During basic research on virus-plant interactions and cell-to-cell movement, PVX was required to express very high levels of an easily detected reporter protein, the 27kDa jellyfish green fluorescent protein (GFP). To achieve expression levels above those from a previous PVX vector which had an additional "promoter" driving an independent GFP gene (analogous to the Biosource Genetics TMV vector), the SCRI group sought to use the native PVC CP subgenomic mRNA promoter to express GFP as an N-terminal fusion to PVX CP. However, they were aware of the earlier TMV and CPMV results, described above, and did not expect that CP subunits of 24kDa fused to the 27kDa GFP would be able to assemble into virus particles, thus preventing virus replication and spread in plants. Therefore, a novel strategy was devised to cleave the fusion protein spontaneously and efficiently (90%) between the GFP and CP domains. A short, 16 amino acid sequence derived from the autolytic FMDV 2A "protease", which disrupts peptide bond formation during translation, was inserted to achieve efficient autocleavage.

However, in infected plants, the modified PVX vector produced not only the predicted free GFP and free CP, but also very high levels of the GFP-CP fusion. In fact, approximately 50% of the CP subunits produced retained the GFP. Surprisingly, these 52kDa subunits assembled efficiently with free CP subunits into PVX particles. The progeny particles were more than twice as wide as wild-type PVX and attained levels *in planta* approaching 10% of the TSP. Indeed, under some circumstances, PVX can attain 0.5 g per kg wet leaf weight! As potential plant factories, the PVX virus vector can infect 240 different plant species including tobacco, turnip and potato; it is not seed- or pollen-transmitted and has no known biological vector (insect, fungus or nematode). Hence it can be biologically contained and does not interact with or transfer its genetic material to the host-plant DNA. Thus, environmental risk issues such as gene flow to wild relative plant species, so topical with genetically engineered transgenic plants, ceases to be an issue with the PVX vector system. It has now been shown that the PVX system can be used to express a range of unrelated proteins ranging in size from 10kDa to 33kDa as assembly competent fusions. Furthermore, the proportion of CP subunits produced as fusions can be modulated through minor modification of the 2A sequence. However, the presence of a small amount of unfused CP is essential for cell-to-cell movement and assembly of the larger OVERCOAT™ fusions.

The development of a high copy number, plant viral vector in which entire large and functional pharmaceutical, therapeutic, industrial or bioremedial proteins, rather than just short peptides, can be expressed as fusions to hundreds or thousands of sites in each final virus particle represents a dramatic breakthrough in plant viral vector technology. At last, the full potential of crop plants as "phactories" to produce valuable proteins, cheaply, safely and rapidly, for a myriad applications can be realised. Plant virus vectors may now supersede "traditional" plant DNA genetic manipulation methods for the construction of green plant factories on laboratory or industrial scales.

b) *Protein quality*

Nutritionally, potato tuber protein suffers from deficiencies in the sulphur amino acids methionine and cysteine. It may be possible to rectify this deficiency by expressing genes encoding methionine /cysteine-rich proteins - for example the 2S (BN 2S) protein from Brazil nut which has a 19% methionine and an 8% cysteine content. With the 35S CaMV promoter Tu *et al.* (1994) showed that

correct processing of the BNS2 gene occurred in transgenic potato petioles, leaves and microtubers although by Western blot analysis, expression in tubers was up to 8-fold lower than in leaves and petioles. Whilst providing no data on protein amino acid composition amino they state that the level of expression observed was insufficient to alter methionine content. They also report mutations in the BN 2S coding region that increase methionine content of the final protein from 19% to 27%. No data are yet available on the protein amino acid composition of plants transformed with the modified construct. However, at the SCRI, Gordon Machray and colleagues have also expressed the brazil nut gene in tubers and preliminary data, with microtubers, indicate a 2- to 3- fold increase in the mole % contribution of methionine to protein amino acid balance. By enhancing expression even further there is every possibility that nutritional value can be improved significantly.

c) *Bruising and Enzymic Browning*

Blackspot bruising of potato results when mechanical damage to the tuber (caused by cutting or rough handling for example) initiates enzymic browning, resulting in the production of black, brown and red pigments. The reaction leading to pigment production is catalysed by polyphenol oxidase (PPO) which converts monophenols to *o*-diphenols and *o*-dihydroxyphenols to *o*-quinones. In potato, the most abundant substrate is chlorogenic acid. A number of PPO genes have been cloned from potato (Thygesen *et al.* 1994). PPO activity has been reduced by gene silencing in potato by the company KEYGENE (and by other groups in the USA and elsewhere). Whilst, following literature database searches we are not aware of any published data on these transgenics, the indications are that the visible symptoms of bruise have been alleviated substantially. Since bruising results from a loss of compartmentation of substrate and enzyme, it seems possible that internal damage to subcellular structures still occurs but without symptom development. Nevertheless, quality for certain outlets could still be affected eg the localised conversion of starch to sugar, which is often associated with cell damage, would still affect the quality of fried products. More detailed information on the performance of these transgenics is awaited.

d) *Novel Carbohydrates*

(i) fructans

Fructans (polyfructosylsucroses) are the dominant carbohydrate in several members of the plant kingdom (though not in potato). They are reported to have a role in tolerance to drought and cold since, unlike starch, they contribute to the osmotic balance of cells. Fructans are also finding a niche in the food industry - they have approximately 10% of the sweetness of sucrose and possess many of the technical properties of sucrose required by the food industry. When ingested they are hydrolysed in the small intestine where they encourage the growth of bifidobacteria, claimed to benefit health. Fructans also yield fructose when hydrolysed. Fructose has been used as natural sweetener in the USA since the 1970's . It is more soluble than sucrose, less viscous, causes fewer dental caries and can be utilised without the need for insulin. Since fructans are synthesised from sucrose (the major translocated carbohydrate in potato) groups are seeking to synthesis commercial quantities in tubers. Thus van der Meer *et al.* (1994) used two bacterial chimeric genes - *sac B* (levansucrase) from *B. subtilis* or *ftf* (fructosyl transferase from *Streptomyces mutans*). Protein production was targeted to the vacuole . The *S. mutans* gene produced fructans with a very high degree of polymerisation (mainly β(2-1) linked with β(2-6) branches. The *B. subtilis* gene produced fructans basically of the β(2-6) type with β(2-1) branches. Transgenic microtubers produced up to 10mg per g FW fructan but the starch content was reduced significantly. Leaves accumulated up to 30% of their dry weight as fructan but again starch content was reduced.

(ii) starches

Starch is the primary storage compound in tubers, accounting for up to 70% of tuber dry matter. Starchy foods are the world's most abundant staples and the nutritional value of starch is supremely important to health. It is the most important source of calories in the animal and human diet and provides a starter material for the preparation of more than 500 different commercial products. A significant proportion of starch produced in Western Europe in used in the food and beverage industries - as a thickener in sauces, custards, pie fillings and desserts. Products of starch breakdown such as maltodextrins, are used in dietetics and low calorie foods or can be used to produce glucose and fructose to be included as a sweetener in drinks or confectionary. In some products starch can be used to replace fat and this will clearly have benefits to human health . Industrially, the glucose produced when starch is completely degraded can be used as a starter material for other processes e.g. the manufacture of vitamins and pharmaceuticals.

The physical properties of starch vary with plant source and also on the source of a particular genotype. The parameters which are of commercial relevance include the number and size distribution of the granules, their amylose and amylopectin content, the extent and distribution of branching within the amylopectin structure and the extent to which the starch is phosphorylated and associated with lipid. Interaction between these influence starch crystallinity , and the physical properties attributed to specific uses and outlets. Given the fact that the potential variation in chemical and physical structure of starch is highly variable there are opportunities to generate novel starches for use in food and non-food market sectors. In particular, the production of novel starches which do not require post extraction chemical processing must be an environmental advantage. This together with the fact that starch is a renewable source, establishes the need to generate new starches and to assess their utility in product development.

The starch granule is built up of two types of glucose-based units. The ***amylopectin*** unit - which is very highly branched, and the ***amylose*** unit - which is branched only slightly. The ratio of amylopectin to amylose is important in defining how the starch is used. The highly branched units give starch its thickening properties. One of the classical success stories in plant biotechnology has been the isolation of a waxy mutant of potato (amf), the identification of the genetic lesion as a mutated granule-bound starch synthase (GBSS1) and the subsequent generation of high amylopectin starch (with no apparent yield penalty) by antisense silencing of the gene (Visser *et al.* 1991 and references therein). So far there has been little or no success in changing starch structure by silencing a related gene (GBSS11) or potato branching enzyme 1 (BE1). However, genes encoding additional synthases and branching enzymes have been or are being cloned from potato and are being used in an attempt to modify starch properties.

Other approaches involve expressing heterologous genes in tubers which code for enzymes capable of linking glucose residues by α(1-4) or α(1-6) linkages. Whilst, in terms of the reaction the catalyse these are identical to potato starch synthases and branching enzymes respectively, the kinetic properties of the enzymes are significantly different. This affects the nature of the polymer produced. Such a gene may be derived from other higher plant species (e.g., pea, wheat), bacteria, fungi, and algae. For example, Shewmaker *et al.* (1994) expressed the *E. coli* glg A gene encoding glycogen synthase in potato tubers. Tubers showed a 30 to 50% reduction in starch content, a decreased amylose:amylopectin structure, a lowered

starch phosphate content and very high branching of the amylopectin fraction. Brabender visco-amylographs and differential scanning colorimetry showed that the starch has a very low stable paste viscosity compared with wildtype controls, reduced enthalpy and gelatinisation properties. The starch resembled cereal starches (*eg* wheat) and behaved in a similar fashion to chemically cross-linked starches. This approach shows clear potential for generating novel polymers.

Carbohydrate metabolism

Tuber physiology and biochemistry is dominated by carbohydrate metabolism and, of all commercial crops, potatoes have become **the** model system for research into to carbon partitioning and source-sink interactions using transgenic plants. Source-sink interactions underpin photosynthetic performance, tuber growth and development, and tuber quality (dry matter content, sweetening and sprouting in storage). It is true to say that our understanding of biochemical and metabolic control is being revolutionised by the application of molecular tools. This understanding will form the platform for a more rational design of strategies to manipulate source-sink relations in the future. Essentially, source-sink relations revolve around sucrose production in the leaf, sucrose loading into the phloem, long-distance transport of sucrose to developing sinks (tubers, roots), regulation of phloem unloading, and last but not least, the metabolic and/or storage fate of sucrose imported into the tuber.
Relatively recent reviews covering work on carbon partitioning in transgenic potato have been published by Sonnewald and Willmitzer (1992), Sonnewald *et al.* (1994) and Frommer and Sonnewald (1995).

i) *Leaf Metabolism*

There are several key elements of carbohydrate metabolism in the leaf which including $C0_2$ fixation, transport of photosynthetically derived metabolites from the chloroplast to cytosol, and the synthesis of sucrose in the cytosol prior to phloem loading. The function of the triose phosphate translocator (TPT) which exchanges photosynthetically derived triose phosphates in the chloroplast with phosphate in the cytosol, has been analysed in antisense transgenics by Riesmeier *et al.* (1993). Transgenics exhibited a dwarf phenotype, starch accumulation in leaves (due to restricted export of triose phosphates) and a reduced capacity of the photosynthetic system. Data indicate that TPT activity can become a rate-limiting factor for maximal rates of photosynthesis. We now await experiments in which

the TPT is over-expressed.

In the cytosol of the leaf the formation of sucrose from photosynthetically derived triose phosphates is considered to be regulated by reactions catalysed by the enzymes fructose-1,6-bisphosphatase and sucrose phosphate synthase (SPS). Antisense suppression of cytosolic fructose-1,6-bisphosphatase to below 20% of activity in the wildtype results in a reduced light-saturated rate of CO_2 assimilation (*ca.* 50% reduction at 1200 μmol photon m^{-2} s^{-1}, quantified by gas exchange at ambient CO_2 levels). However, sucrose concentrations remained unaffected in source leaves of transgenic plants whilst starch levels increased up to 3-fold in the light (Zrenner *et al.* 1996). Despite the fact that down-regulating bisphospatase activity induces significant alterations total in carbon partitioning within the leaf, there is no obvious effect on tuber yield (both in terms of fresh weight per plant and % dry matter content). It appears that the transgenic lines mobilise the additional leaf starch, formed by diverting a proportion of metabolism away from sucrose, at night. However, in the dark it is likely that hexoses or hexose phosphates are exported out of the chloroplast since the reduced activity of the biphosphatase should preclude significant sucrose biosynthesis from triose phosphates (which are exported from chloroplasts in the light). This clearly demonstrates that the potato plant is capable of modifying its carbon allocation patterns and that restricted rates of sucrose biosynthesis need not lead to adverse effects on yield (at least in glasshouse-grown plants).

Down-regulation of SPS in potato leaves inhibits sucrose formation and stimulates starch production (Krause, 1994 cited by Frommer and Sonnewald, 1995). However, SPS exists exist in phosphorylated and non-phosphorylated states which affects the catalytic activity of the protein. SPS activation state was enhanced in antisense plants, indicating that the plant attempts to compensate for lower SPS protein content by regulating activity *per se*.

ii) *Phloem loading*

Heinke *et al.* (1992) provided convincing evidence that the loading of sucrose into the phloem of potato leaves involved an apoplastic step. They expressed a yeast invertase in the cell wall which resulted leaf bleaching, reduced root growth and tuber yield. This sucrose must enter the cell wall free space prior to loading. Evidence for a loading step involving a sucrose transporter came with transgenic potato with down-regulated expression of the sucrose transporter gene StSUT1

(Riesmeier *et al.* 1994; Kuhn and Frommer 1995) . Again, plants were retarded in growth, exhibiting a much reduced capacity for sucrose transport.

iii) *Phloem unloading*

Potato tubers have a high frequency of plasmodesmatal connections, suggesting that symplastic unloading, along a mass flow gradient, is the primary unloading route. This fits with the observation that tuber-specific inhibition of the sucrose carrier only leads to a slight reduction in tuber yield (leaf phenotype is normal - Kuhn and Frommer, unpublished data cited by Frommer and Sonnewald). The fact that ectopic expression of a yeast invertase in the potato cell wall appears to increase sink strength and tuber yield argues that at least part of the incoming sucrose may be unloaded apoplastically.

iv) *Sink strength*

sucrose breakdown
Sucrose hydrolysis can be regarded as the first metabolic step once sucrose is unloaded into the storage parenchyma cells. Sucrose synthase (SUSY) is the predominant sucrose-degrading enzyme in developing tubers actively synthesising starch. Zrenner *et al.* (1994) demonstrated that down-regulating SUSY inhibits starch accumulation, reduces yield, elevates hexose levels but has no effect on sucrose content (in direct contrast to the effects observed when another enzyme on the sucrose to starch pathway, ADPglucose pyrophosphorylase is down-regulated - see below). The increased hexose content is paralleled by substantially increases in invertase activity, presumably as the plant attempts, in vain, to compensate for reduced SUSY activity. Trangenics also showed a reduction in the level of soluble tuber proteins. Such pleiotropic effects are not unusual in transgenic plants and serve to indicate the plastic nature of plant metabolism and the need to assess the biochemistry of the transgenics adequately. The SUSY transgenics indicate that control of sucrose breakdown is important to sink strength development. This is supported by the observation (see Frommer and Sonnewald 1995) that tubers expressing yeast invertase in the cytosol show a 30% increase in total fresh weight per plant (tuber size increased but tuber number declined).

Transgenic tubers with down-regulated expression of vacuolar invertase showed little change in tuber yield, confirming its subordinate role in regulating sink

strength in developing tubers (Zrenner 1996). Hexose levels were reduced in antisense plants following 20 weeks at 4°C (from 75 μmol g FW to 50 μmol g FW) whereas sucrose remained higher in transgenic tubers compared with wildtype control (41 μmol g FW compared with 28 μmol g FW). Given the fact that sucrose synthase is not normally found in stored tubers and that sucrose cleavage to reducing sugars (hexose) is mediated by invertase, it is somewhat surprising that reducing invertase activity to only 8% of wildtype has such a small effect on sweetening. These data clearly suggest that sweetening is a polygenic trait in which control is exerted at several points in the pathway from starch through sucrose to hexose. However, the vast majority of published data on tuber yield , numbers *etc* is drawn from transgenic plants grown in pots under greenhouse conditions. It is imperative that adequate field trialling is completed before we can draw any conclusions on the positive impact of transgene expression on sink strength and yield.

starch biosynthesis

The major regulatory step in starch biosynthesis within the starch-storing plastid (amyloplast) is the conversion of glucose-1-phosphate to ADPglucose catalysed by the enzyme ADPglucose pyrophosphorylase (ADPG PPase). This was superbly demonstrated by Muller-Rober *et al.* in 1992 following the silencing of ADPG PPase activity in transgenic plants. Tubers containing only 2 to 5% of wildtype ADPG PPase activity contained only 2 to 5% starch but the sucrose content increased to 30% of the dry weight, glucose increasing to 8%. This dramatic manipulation of sink strength also resulted in a 10-fold increase in tuber number, interpreted as a response to a major decline in the sink capacity of individual tubers.

In another milestone paper, Stark *et al.* (1992) at Monsanto produced transgenic tubers of Russett Burbank with a starch content elevated by up to 30% by over-expressing an *E. coli* ADPG PPase in tubers. The *E. coli* gene was a mutated form which encoded a protein insensitive to the allosteric regulators 3-PGA and P*i* which regulate the activity of the native plant enzyme. Similar effects with other cultivars have been observed following transformation with ADPG PPase from barley which also has modified allosteric properties (Peter Poulsen, personal communication). At the SCRI we have confirmed that the Monsanto plants have an increased dry matter content and show an increased potential for converting radiolabelled precursors into starch. Perhaps due to the longevity of the *E.coli* protein, significant ADPG PPase activity is also detected in stored tubers. The

ability of these transgenics to cycle carbon from starch breakdown products back into starch may explain why low-temperature sweetening is reduced in this material and why the tubers respond well to re-conditioning (unpublished data).

The future

Future challenges include the identification of new promoters e.g. to drive expression in stored and sprouting tubers, new sources of genes for resistance and quality traits and perhaps the need for a wider range of more "acceptable" selectable markers not based on antibiotic resistance. New vectors and selectable markers will be important if traits are to be modified by over-expressing or antisensing several genes simultaneously. Scientists must also begin to address the problems of selecting commercially viable transgenics under glasshouse conditions, since this does not appear to be a substitute for selection in the field. The effects of environment on transgene expression and trait development and stability will need to be addressed.

- **Release of Genetically Modified/Manipulated Organisms (GMOs) and their Products to the Environment and for Foodstuffs**

To a large extent there is relatively little opposition by the public to the use of microbially-based genetic engineering in most areas of health care, but as the technology is finding application in food production, storage and processing there is widely expressed consumer concern in Europe especially about safety. Transgenic mammals evoke strong reactions in the media and by pressure groups. Some GMOs and their products will continue to be contained strictly within the laboratory environment, others will be monitored closely over a long period to examine their suitability for release into the environment and for consumption. A case-by-case analysis should be carried out in every instance.

Safety is not absolute thus the nub of the argument is one of setting the level of acceptability of risk. By way of definition, hazard is a situation that may lead to harm or loss: risk is the chance in quantitative terms of a defined hazard occurring.

Biologists appreciate that there is natural transfer of genes controlling the formation of toxic principles in plants as well as desirable features. For the most part, species are not static genetically. Through natural selection, for example, resistances to a range of adverse xenobiotics and biotic stresses can be developed.

One huge problem is that the forecasting performance "in the field". We are not knowledgeable about selection pressures operating on organisms, nor about unique recombination events likely to occur. Frankly, it is not feasible for any committee or individual to assess risk for all the possible combinations of genes. Assessment can only come from experimentation and monitoring, employing a battery of scientific disciplines. To date, release experiments have not been problematical. Even naturally occurring mobile DNA elements are limited in their natural hosts, and are not excessively promiscuous. Wilson (1993) cites that an analysis of 393 defined field trials of transgenic plants (25 species) between 1986-1991 (in 21 countries) revealed that 50 involved "virus-resistance" traits. Field releases have shown that coat-protein-mediated protection may not behave as predicted in laboratory and growth-chamber experiments; generally, there is greater **susceptibility** to virus challenge, possibly due to faulty experimentation.

The use of selectable marker genes, especially those that might have environmental and health impacts *eg* antibiotic resistance, raises questions of safety. The potential for uncontrolled gene transfer in the gastro-intestinal tract, soil or by cross-fertilization, or for example herbicide resistance leading to the creation of weeds have received the most attention. What information is available would not indicate unacceptable risk. Obviously, a great deal of research is still required to quantify risk, if any, and to make recommendations on the use of marker genes. With time, their use will in any case decline; essential markers may need to be inactivated or eliminated prior to release or consumption of the transgenic organism.

Organisational structures to monitor GMO release are already in place in many countries. Much of the real work, though, is labour-intensive, from removal of flowers to stop breeding of transgenics with other plants (especially weeds), surveying experimental sites to eliminate propagules (seeds, tubers *etc*) in the seasons following the experiment, and monitoring gene flow through ecosystems. It is always a good policy for any country to monitor its flora and fauna anyway. At SCRI, R J McNicol and colleagues have been monitoring gene flow in fruit crops using non-GMO marker genes for 39 years. We are deeply suspicious about requirements for "analysis of benefit" prior to permission being given for the release and use of GMOs. Just who sets the criteria of benefit and performs the analysis? Central planning can be debilitating when prudency and responsible care are the objectives. We can contrast the constraints on the release of GMOs with the far less stringent monitoring of pesticide residues in foodstuffs.

- **<u>Public Attitudes</u>**

Voters comprise heterogeneous groups who determine the political, industrial and economic climate of democratic countries. Their taxes and those of private companies support R&D programmes in the public sector. They are also consumers who should be free to exercise choice. Scientists should be providing them with factual basis for reaching informed decisions.

In contrast to healthcare, applications of modern biotechnology to food and the environment are greatly influenced by the level of education, perceived social and ethical issues, as well as reaction, frequent irrational, responses towards non-medical sciences.

There are also objections at a secondary level to the role of multinational companies carrying out genetic engineering and failing to take adequately into account the impact of their activities on the less-developed world, playing one economy off against another, or riding roughshod over the need to label products. Although not necessarily associated with religious organisations, there is also the oft-cited "unnatural" or "ungodliness" aspect of science replacing natural functions, generating chimaeric organisms, or fiddling with life for profit. With respect to the less-developed world, the reality is one of enthusiasm for biotechnology which represents relatively low-cost advanced technology easily translatable to the urgent needs of those seeking wealth creation and quality of life objectives.

Ignorance of science and technology, as much as ignorance of business, leads to fear, anxiety and reluctance to fund research and development projects. Pressure groups of all kinds are formed. It seems that the public derive most of their limited understanding of science through the arts-dominated media, especially television, where all too often artistic license embellishes scientific observation with imaginative doom-laden claptrap. This must cause the scientific and advanced industrial communities a measure of introspection. Healthy scientific scepticism, questioning, sharp debate, experimentation and wide-ranging open-mind interpretations and conclusions are the stuff of science. So is presentation. There can be no room for indolence nor ineptitude. Scientists, like the public, cover a spectrum of views and attitudes and are difficult to organise except into cliques. The public must realise that modern civilisation is entirely technology-dependent. Scientists in turn accept justifiable control as much as the financial backing. We are worried that the foucault pendulum is swinging towards harsher controls which cannot be sustained in the longer term even though it is unfashionable to argue against any moves restraining science. Sometimes, pressure groups have vested political interests or social engineering at heart. Nonetheless, a balance-point must be reached, taking into account illogical fears, damage to the environment,

healthcare, and the need for science. The need for genetic engineering is irrefutable. Indeed, the UK Technology Foresight Programme (now termed Foresight) has highlighted the key rôle of modern biotechnology for wealth creation, quality of life and industrial competitiveness, and this conclusion has also been reached in other national foresight programmes. It is how we deliver biotechnology that we must get right. Clearly, full labelling of GMO products is essential, as is that of the inputs of commercially produced material. Information overcomes the prostitution of ignorance.

At present, there is a view that only by being aware of obvious benefits to the consumer (*eg* increased safety because of reduced natural toxins, lower costs, pest- and disease-free produce, better and more consistent quality *etc*) or to the environment (*eg* reduced pesticide inputs, bioremediation *etc*) will there be general acceptance to genetic engineering. Fewer problems are experienced with plant-plant than with plant-microbe transgenics; plant-animal and animal-animal combinations, most notably where "human" genes are concerned can provide virulent public and pressure group reactions. The greatest level of acceptance seems to favour transgenic plants used for non-food purposes. Indeed, transgenic potatoes modified for starch structure now in commercial production in the Netherlands. There is also considerable investment by sugar-beet breeding companies for the release of transgenic sugar beet. Meanwhile, there will be a plethora of legislative barriers. In the medium to long term, however, the imperative to acquire food to feed an extra two billion mouths before 2025 will create the demand for the benefits of plant biotechnology.

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