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MOLECULAR AGRICULTURE: PROSPECTS FOR THE PRODUCTION OF ALTERNATIVE COMMODITIES IN SUGARCANE THROUGH GENETIC ENGINEERING

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Abstract

The production of crop plants capable of synthesising new compounds is now possible due to recent advances in genetic manipulation techniques. Since plants can be transformed with genetic material from virtually any source, the number and range of potential products is extensive. Examples investigated to date include unusual carbohydrates, unsaturated fats, protein sweeteners, vaccines for important diseases, drugs and plastics. Most of these products have been developed in 'model' crops such as tobacco, tomato, potato and alfalfa. Although few novel compounds are in the commercial production phase, the greatest impact of the technology has been in the food additive, specialty chemical and pharmaceutical industries. In this paper it is argued that, to develop an appropriate genetic engineering strategy and to make a realistic appraisal of its practical implications, detailed knowledge of the genetic and metabolic control of the product of interest, as well as the biochemistry of the crop plant, would be prerequisite. Strategic options are presented for both genetic and metabolic manipulation, and their general significance for subsequent processing of the product in sugarcane is discussed. A number of general problems are highlighted: the patenting trend, the resultant lack of readily available genes, economic constraints and the need for international regulatory procedures.

Introduction

During the past century many attempts have been made to develop sugarcane by-products as useful and saleable commodities. For example, a method for cane wax recovery was patented by A Wijnberg in 1909; by 1916 cane wax was being produced commercially in South Africa and, in 1924, it was reported that the export from Natal alone was 6 000 tons annually (Paturau, 1989). Despite some initial successes, most such ventures ultimately collapsed. Although in a few cases this may have been due to changes in market demand, it is reasonable to conclude that most failures were the result of either an envisaged product being absent in sugarcane or a known product being present in too low a concentration. Both of these factors reflect the genetic potential of the crop. It is probable that the desired characters could not be achieved through conventional breeding, since the genetic base of sugarcane is thought to be fairly narrow (Berding and Roach, 1987). Hence the only realistic route leading to the genetic changes required for either by-product or novel product development in the future would be that of genetic engineering.

In the past decade it has become increasingly evident that the techniques of genetic manipulation allow both the enhancement of endogenous products in crop plants and new product development by the insertion of novel (foreign) genes. It is also clear from internationally published and in-house research that sugarcane is amenable to genetic engineering approaches.

Sugarcane is a crop with a particularly high above-ground biomass, 30% of which never reaches the millyard (Thompson, 1991). Clearly, introducing genetic alterations which would make this 'waste material' useful is an attractive concept. However, it is not the intention of this paper to cover the potential of modifying biomass to realise a large scale/low return product, but rather to focus on genetic alterations that can lead to novel biochemical products of moderate to high market value. The paper highlights a wide range of examples of biochemical commodities currently being developed, through genetic engineering, as potential agricultural products in various crops. It also attempts to pinpoint some of the key factors that should be considered before any initiative is taken to establish a biotechnological programme for the production of alternative commodities in sugarcane. It is not intended to present a comprehensive review of the available literature, nor does it take market related (financial) issues into detailed consideration.

Examples of alternative commodities

In the literature of the past twenty years, there are many well documented examples of economically important compounds that can be produced in practicable quantities by genetically engineered microbes, unicellular algae and plant cell cultures. It is only more recently, in parallel with the proliferation of genetic manipulation technologies applicable to higher organisms, that the potential of livestock and crop plants to generate unconventional products of interest has been exploited. The increased use of farm animals for the commercial production of pharmaceutical products in particular has stimulated the term 'pharming' (Spalding, 1992), but plants have been targeted as potential factories for a much wider range of compounds. Apart from being directly useful as sources of food and fibre, specific plants may be natural producers of oils and fats, polymers, industrial chemicals, dyes, detergents, medicines and cosmetics. Thus the potential to enhance the productivity of a particular biochemical compound in a crop by genetic engineering has been viewed as important by a spectrum of industries. The greatest impact of the technology to date in the commercial marketplace has been in the fields of food additives, specialty chemicals, drugs and other pharmaceutical products (Fraley, 1992).

Since the emphasis of this paper is on the potential of sugarcane as a manufacturing plant, the selected examples of alternative commodities given in Table 1 are limited to those involving higher plant production.

Appropriate genetic engineering strategies

For the purpose of genetically engineering a crop plant that will meet a specific production need, it is essential to devise an appropriate strategy. Genetic engineering strategies that can be used to modify plant function fall into three categories: (1) up- or down-regulation of endogenous gene expression, (2) modifications to endogenous genes and their products and (3) introduction of exogenous genes into the

Table 1

Selected examples of alternative products developed in crop plants by genetic engineering.

Product	Product category	Specific use	Crop plant species	Strategy	References	Comments
Fructan	Carbohydrate	Low calorie sweetener Nutrient source of fructose	Tobacco Potato Chicory	Bacterial transgene Antisense	Ebskamp *et al.*, 1994; van de Meer *et al.*, 1994 Brown, 1992	
Trehalose	Carbohydrate (disaccharide)	Nutrient sweetener Preservative	Sugarbeet, Potato Tomato	Bacterial transgene	Kidd & Devorak, 1994	Commercial companies involved: Mogen Int., Osmotica Foods, Van der Have
Cyclodextrins	Carbohydrate		Potato		Oakes *et al.*, 1991	
Unsaturated fatty acids	Lipids	Nutrient	Tobacco	Stearyl-CoA desaturase transgene from rat	Grayburn *et al.*, 1992	Fatty acid alteration
Stearoyl-acyl carrier protein desaturase	Lipids	Induces double bonds in stearoyl-acp	Rapeseed	Antisense	Knutzon *et al*, 1992	
Glycerol-3-phosphate acyltransferase	Lipids	Reduces unsaturated fatty acids	Tobacco	Enzyme	Murata *et al.*, 1992	
Monellin	Protein (heterodimer)	Sweetener/flavorant (non-carbohydrate)	Lettuce, tomato	Transgenes from other plant species	Peñarrubia *et al.*, 1992	100 000 × sweeter than sugar
Ricin	Protein	Immunotoxin in cancer therapy	Castor bean	Transgenes	Spooner & Lord, 1990	
Ribosome-inactivating proteins (RIPs)	Protein	Antiviral and abortifacient activity	Corn, barley, wheat, asparagus	Transgene	Stirpe *et al.*, 1992	
α-Amylase	Protein	Starch liquefaction	Tobacco	Transgene	Pen *et al.*, 1992	
Antibody	Protein	Vaccines against malaria, HBV, Foot and mouth disease	Tobacco and others		Coghlan, 1995	Estimated 250 kg vaccine per month per hectare
		Identification of blood groups	Alfalfa	Transgenes	Khoudi *et al.*, 1994	
Human Protein C	Protein	Anticoagulation in blood stream	Tobacco	Transgene	Piché & Fortin, 1994	
Phytase	Protein	Increases availability of phosphorus from feed for monogastric animals	Tobacco	Transgene from *Aspergillus niger*	Verwoerd *et al.*, 1994	
Starch synthase	Protein	Inhibits starch synthesis	Potato	Enzyme	Visser *et al.*, 1991	
Lysine, methionine, tryptamine	Amino acid	Additive to enhance animal feed quality	Tobacco Soya beans	Transgenes	Shaul & Galili, 1992; Songstad *et al.*, 1990; Beck & Ulrich, 1993	
Phytoalexin	Secondary product	Antifungal agent	Chickpea	Altered enzyme regulation	Marsh, 1990	
Carotenoids	Secondary product	Pigments			Bartley *et al.*, 1994	
Biodegradable plastic	Secondary product	Plastic	Oilseed rape	Poly-β hydroxybutyrate bacterial transgene	Smith *et al.*, 1994	Marketed as "Biopol"
Latex	Secondary product	Latex products	Opium poppy	Latex protein transgenes	Nessler *et al.*, 1990	

plant. To assess these strategic options and their limitations, knowledge of fundamental molecular genetic principles and understanding of certain genetic and metabolic features of the particular product of interest are essential prerequisites.

A basic outline of gene function is given in Figure 1. Proteins are the first functional molecules to result from gene expression and thus their synthesis is linked directly to genetic (DNA and mRNA) activity. However, the importance of many of the proteins lies in the fact that they are enzymes which catalyse reactions leading to other biochemical compounds. Hence a compound that requires a complex chain of enzymatic reactions for synthesis is indirectly linked to, and dependent on, the activity of a number of genes. In the simplest scenario, where it is desired to isolate significant quantities of a simple protein product, identification of the gene(s) involved in protein production would be required before a strategy could be devised to induce overexpression in the plant. In some cases, knowledge of the genes encoding the relevant control factors (Figure 1) might also be necessary. Where a complex secondary product is of commercial interest, knowledge of the metabolic pathways leading to the product would be a prerequisite, and it would be essential to identify the rate limiting enzymatic step(s) and the gene(s) controlling the enzyme(s) involved, before designing a manipulation strategy.

Up- or down-regulation of endogenous gene expression

In the simplest and most common approach, up-regulation of the expression of a gene already present in the plant can be achieved through the introduction of multiple copies of the gene, particularly if they are constructed to be adjacent to a 'strong' promoter (regulatory element which drives expression). Alternatively, up-regulation can be achieved by enhancing the expression of relevant transcription activators when the genetic control of these is known. Inhibition of gene expression can also be done, either directly, using antisense technology, or indirectly by manipulating transcription factors. The antisense technique involves the insertion into the plant of a DNA sequence which is in reverse orientation to the gene sequence (sense message) to be suppressed. For example, a gene that encodes a catabolic enzyme of a pathway can be inhibited using this approach, resulting in an increase in the amount of metabolite that is normally broken down. The strategy of biochemical pathway manipulation is sometimes termed 'metabolic engineering'.

Modifications to endogenous genes and their products

In cases where the mechanistic basis for a specific gene product is known, useful changes may be made by changing endogenous gene sequences to encode proteins with slightly altered characteristics – for example, enzymes with modified substrate specificities or allosteric properties, which will show abnormal metabolic responses. It has been shown, for instance, that introduction into potato of a gene for feedback-insensitive ADP-glucose pyrophosphorylase, which catalyses the first step of starch biosynthesis, resulted in a commercially significant increase in starch accumulation in the tubers (Gibson and Somerville, 1993).

Introduction of exogenous genes into the plant

The use of genes from external sources - other plant species, microbes or animals - to enhance endogenous gene

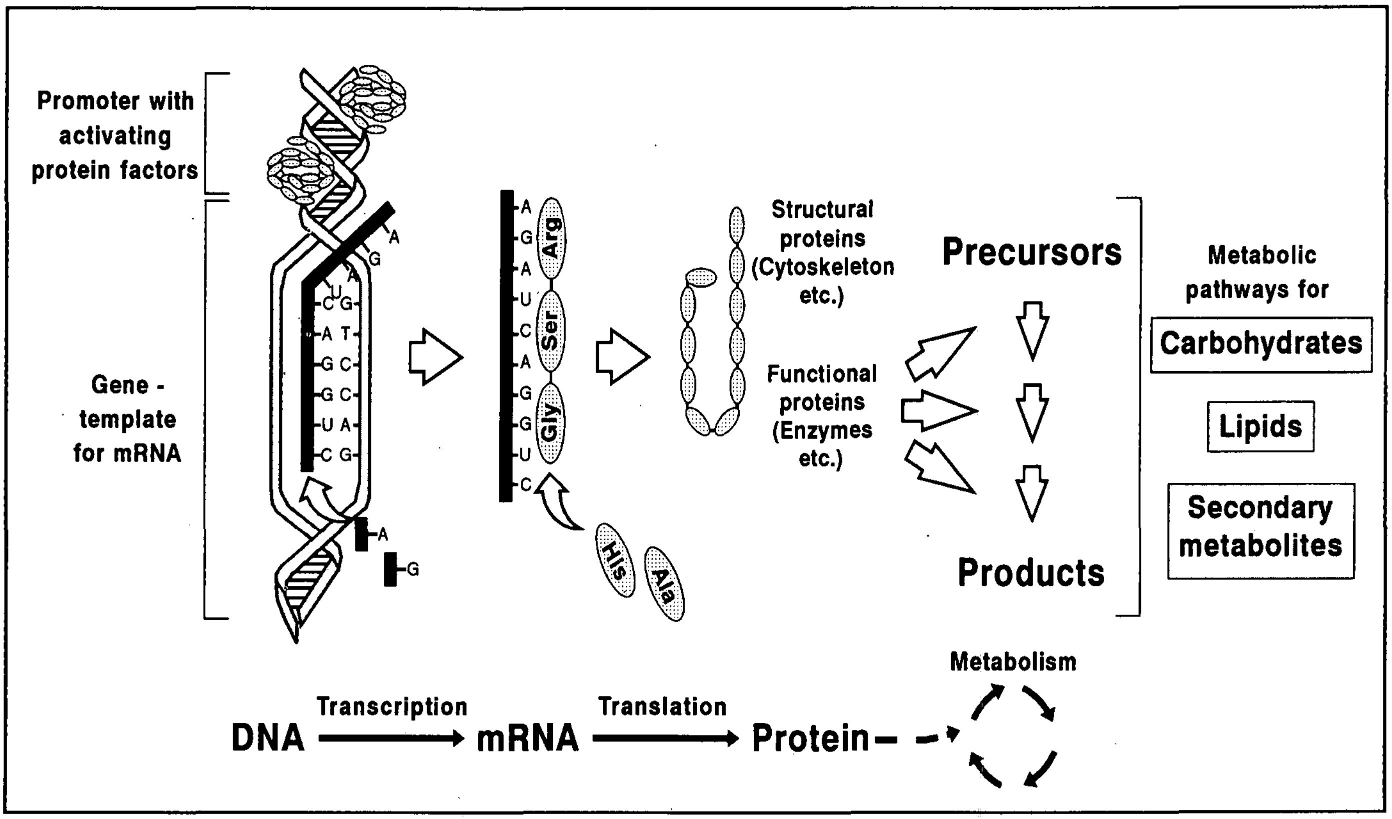

FIGURE 1 Flow of genetic information from DNA through RNA to proteins. Deoxyribonucleic acid (DNA) is a double stranded long-chain polymer composed of four nucleotide building blocks -adenine (A), thymine (T), guanine (G) and cytosine (C). The specific sequence of these nucleotides is the repository of all genetic information and is used to synthesise all the proteins of the organism. Protein synthesis occurs in two stages: 1. During *transcription* a defined stretch of DNA, a gene, serves as template for the synthesis of a complementary single stranded messenger ribonucleic acid (mRNA); 2. In a second step the linear sequence of nucleotides in the mRNA is *translated* into a linear sequence of amino acids to form a polypeptide chain which is the basic structural unit of proteins.

activity or produce novelty biochemicals in crop plants, has massively increased the wealth of possibilities available. Microbial genes are identified and isolated more easily and quickly than endogenous plant genes, so the strategy of using a microbial gene to up- or down-regulate an endogenous homologue is often employed. In other cases, microbial genes or genes from other plant species which control the production of obscure compounds, not found universally, are the means of introducing useful novel products into crops. The majority of microbial and plant products used as drugs and dyes, for example, have evolved in a limited taxonomic group of organisms, often in a single species, and the isolation and transfer of the relevant genes to highly developed crop plants is the basis of many current examples of alternative commodity research. In some cases, but by no means all, the exogenous gene of interest would have to be modified to obtain expression in the crop. In all cases it would be essential to link the gene to an appropriate promoter.

An overview is presented (Figure 2 which places the genetic engineering strategies discussed above in the wider context of commercial research and development).

The importance of physiological knowledge of the crop: fine-tuning strategy

As mentioned previously, an understanding of metabolism is an important prior condition for selecting an appropriate alternative product. The importance of this is best illustrated by the inability to use modern technology effectively to manipulate sucrose accumulation in sugarcane. This is due to our limited understanding of the biochemical processes responsible for sucrose accumulation (John, 1992). The introduction of a novel product into sugarcane would have to be prefaced, therefore, by a better definition of background biochemistry to safeguard against unpredicted consequential metabolic changes. However, even with limited knowledge, it can be speculated that the introduction of a new product will influence the regulation of existing metabolism. The following discussion briefly highlight some of the important issues regarding metabolism and carbon partitioning in sugarcane.

Carbon partitioning

The amount of carbon dioxide fixed by harvesting sunlight energy probably varies little between existing sugarcane varieties (Irvine, 1975). Once fixed, the carbon is used to sustain a variety of metabolic pathways (Figure 3) in the different tissues. However, the biomass produced, and the fibre and sucrose contents, can differ substantially between varieties. It can be assumed, therefore, that the efficiency with which the fixed carbon is partitioned between the different metabolic pathways, i.e. carbon partitioning, must differ. The partitioning of compounds in the plant is driven by a sink/source relationship, where the tissue primarily responsible for the production of a compound is referred to as the source and the tissue with a net import, the sink. It is evident, for example, that increased synthesis of protein will place an additional drain on the amino acid pool. Consequently, through a cascade effect, carbon will be drawn from several pathways and ultimately from sucrose. In addition, a further load will be placed on energy supply and nitrogen availability (Figure 3). Following the principle of sink/source relationship it is clear that the production of a new compound, even in a distant tissue, will upset the carbon partitioning in the plant.

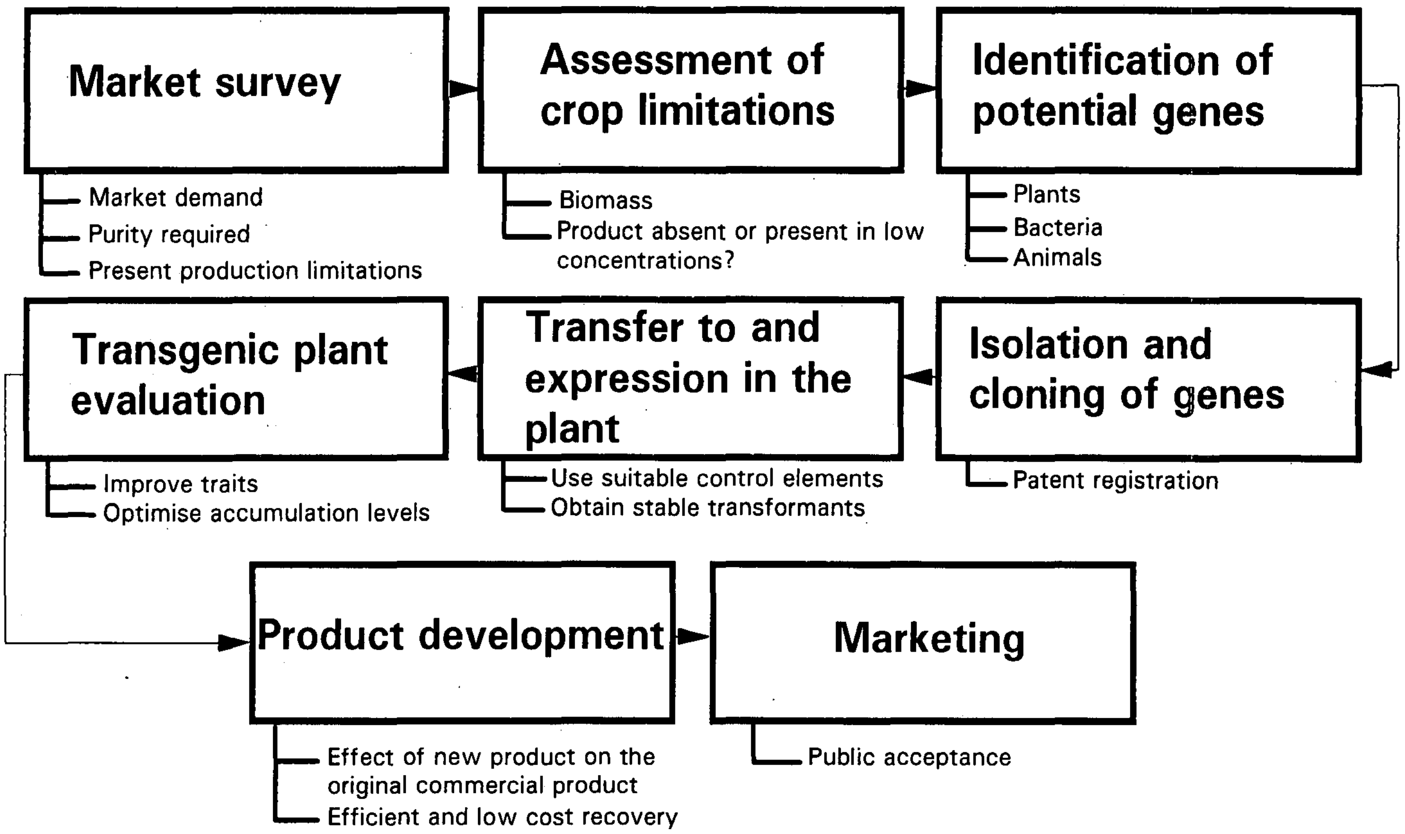

FIGURE 2 Scheme showing the sequence of research and development steps required for commercial production of an alternative crop plant commodity.

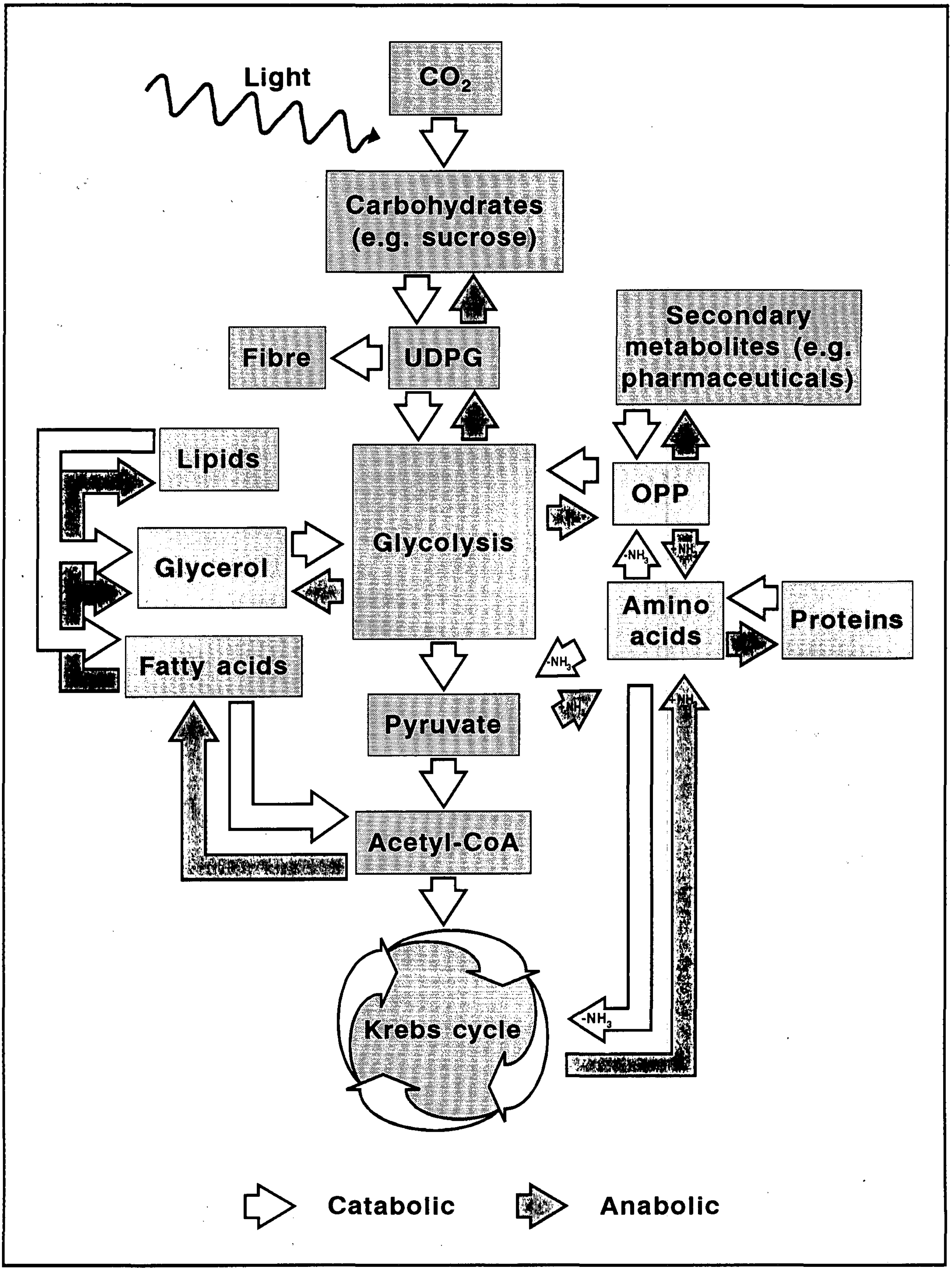

FIGURE 3 Simplified diagrammatic representation of carbon flow between the major metabolic pools in plants. UDPG = UDP-glucose. OPP = oxidative pentose phosphate pathway.

Strategic option: targeting the product to a specific part of the plant

From the foregoing argument it should be obvious that the production of large quantities of an alternative product, in addition to retaining present levels of sucrose, might be very difficult to achieve. However, the smaller the additional load on carbon and energy, the better the chances of retaining present sucrose levels. Two logical approaches would be to limit production of the new commodity by targeting it to specific tissues and/or to concentrate on high priced products. Although targeting is possible, and in fact has been used successfully in certain crops, our knowledge of tissue-specific genetic regulatory elements for sugarcane is limited at present. However, available knowledge suggests that, with the necessary input, targeting of a new product in sugarcane will be possible.

The effect of targeted products on processing

Apart from the advantage of product targeting in reducing the negative effect on carbon and energy demand, it should also be considered in relation to respective recovery efficiencies of the additional product and sucrose. For example, if the new product was water soluble and a non-protein, it might cause contamination problems in the recovery of sucrose unless its synthesis could be restricted to non-stalk tissue. In the case of proteins, especially for pharmaceutical use, it would be advantageous to target accumulation to the outside of the cell as this would greatly reduce purification costs. A further consideration is that the nature of the target tissue expressing the new trait would be the major factor in determining whether up- or downstream processing in the present milling system could be exploited, or whether a totally new processing plant would be required.

Other considerations

Availability of useful genes and the patent problem

Those genes that have been isolated to date worldwide represent an extremely small proportion of the total number available in living organisms. This means that there is enormous potential for future biotechnological developments based on novel genes. As a result, the process of gene isolation is in an exponential phase of growth, and the number of registered gene sequences is increasing daily. However, of those genes isolated, only a few will have implications for the production of alternative products in plants. Furthermore, most of the genes that are useful in this regard have been isolated by commercial companies and are protected by patent registration. It is significant that only a small number of reports in this field of agritechnological development are published as open scientific papers at major conferences or in international journals. Much of the relevant information is not published in full, but takes the form of commercial press releases, editorial commentary or unreferenced reviews. The inference is clear: the sugar industry will not be able to assume access to information or sequences, and any proposed endeavour in this field will have to include preparatory steps such as the isolation of appropriate genes and regulatory elements before genetic manipulation of the crop *per se* is possible.

Economic implications and constraints

Molecular modification of sugarcane will require extensive research to investigate and ultimately obtain the desired genes and regulatory sequences, optimise gene transfer and characterise the resultant transgenic plants. As with any other transgenic work, field performance trials would be essential to investigate the effect of both the transformation and regeneration processes, as well as the expression of the new product on the agronomic performance of the crop, before moving to commercial application (Krebbers *et al.,* 1993). In terms of industrial development, it must be ensured that processing is compatible with the extraction and purity requirements of the alternative product and that production and purification costs are in turn evaluated in light of the average product yield and growing costs (Willmitzer and Töpfer, 1992; Pen *et al.,*1993). For example, savings related to harvesting could offset the effective cost of a lower product yield, whereas a high priced product might involve costly processing.

Existing barriers to commercialisation

One of the main obstacles blocking commercialisation of 'manufacturing plants' is regulatory approval (Fraley, 1992). Gaining the appropriate approval is critical for product development (Fuchs and Perlak, 1992). Although this has stimulated collaboration between a diversity of industries and regulatory agencies, there is still a requirement for science-based harmonisation of regulatory requirements (Fraley, 1992; Fuchs and Perlak, 1992). A globally acceptable and enforced set of regulations is likely to be in place in the next few years. In the meantime, South Africa recognises the principles laid down by the South African Committee for Genetic Experimentation (SAGENE), which in turn are based on those of the USA.

Conclusions

There is worldwide interest in the concept of crop plants as producers of non-traditional commodities. Genetic engineering is sufficiently well advanced that a number of ideas in this field have been followed through to the point where transgenic plants generating alternative products have been produced and assessed. However, few of those successes are in the commercial production phase as yet. There is a strong tendency for research and development in this field to be closed, and both useful genes and transgenic products to be patented. Venture into this area in the sugar industry would require considerable capital investment and time, as there would be the need to identify and isolate genes of interest, as well as the need to extend knowledge of sugarcane metabolism before embarking on any transformation strategy. From the beginning of any enterprise of this nature, collaboration between research workers in marketing, growing and processing would be essential for success.

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