Production of novel polymers in transgenic plants

Jörg Riesmeier, a Jens Kobmann, b Richard Trethewey, b Arnd Heyer, b Volker Landschütze a & Lothar Willmitzer b

aPlantTec Biotechnologie GmbH, Hermannswerder 14, 14473 Potsdam, Germany
bMax-Planck-Institut of Molecular Plant Physiology, Karl-Liebknechtstr. 25, 14476 Golm, Germany

(Accepted 4 August 1997)

The production of novel or modified polymers in transgenic plants can be divided into the manipulation of plant metabolism to modify in planta endogenous polymers and the introduction of metabolic pathways from other plant species or even bacteria into crop species to produce novel polymers. Polymers of interest are starch, fructans and polyhydroxyalkanoates (PHA). During the last few years several genes involved in starch biosynthesis have been isolated. Following the antisense or the overexpression approach transgenic plants were created which produced starches displaying altered physical and chemical properties. The isolation of genes necessary for the synthesis of fructans from agronomically unfavourable plant species will allow the production of this polymer in a set of transgenic crops on a reduced cost level. The production of small amounts of PHB in transgenic plants was demonstrated some years ago. For commercial production of PHB it is necessary to further increase the capacity of transgenic plants to synthesise and store PHB and study the possibilities to manipulate metabolic pathways of the plant for the production of other PHA precursors.

© 1998 Elsevier Science Limited. All rights reserved

1 INTRODUCTION

In the next two or three decades the production of plant-based materials will have to be raised dramatically due to a world-wide increase in the human population and changes in consumer behaviour. On the other hand, the total expanse of land under cultivation will remain constant or may even decline. Farmers, breeders and the agrochemical industry have to face this challenge together. It is now necessary to evaluate and use all available traditional and new techniques to increase crop yield on an ecologically and economically favourable basis.

Obviously, transgenic plants will play a crucial role in modern agriculture. Crops displaying herbicide or insect resistance were grown world-wide on several million hectares in 1996. This first generation of transgenic crops will be complemented by the introduction of characteristics such as increased yield, altered oil composition and novel or modified polymers. In this paper we will discuss the current status of the production of novel or modified polymers in transgenic plants.

2 MODIFIED STARCHES IN TRANSGENIC PLANTS

More than 6 million tons of starch per year are currently produced in Europe and used in a wide range of different industries. Native starch is normally composed of essentially linear (0.1% branchpoints) \( \alpha \)-1,4-glucans (amylose) and via \( \alpha \)-1,6-glycosidic bonds branched (4–5% branchpoints) \( \alpha \)-1,4-glucans (amylopectin). The major sources are maize, wheat and potato. Approximately 30% of starch is used in its native form, and 15% in a chemically modified form. These chemical modifications are normally introduced to optimise the physicochemical properties of the different starches for industrial applications, such as an adhesive in paper and textile manufacturing.¹

Genetic engineering of plants might serve as a tool to replace some of the chemical modifications, if it is possible to manipulate key steps of starch biosynthesis, which are of major importance in determining certain properties of the starch synthesised in transgenic plants.
Starch is synthesised through the ADP-glucose pathway, involving the three enzymes ADP-glucose pyrophosphorylase, starch synthase and starch branching enzyme. ADP-glucose pyrophosphorylase is the key enzyme of the pathway, determining the flux of carbon into starch. It generates ADP-glucose, which is the substrate for the starch synthases, from glucose-1-phosphate and ATP releasing pyrophosphate. The enzyme is stimulated by 3-phosphoglycerate and inhibited through inorganic phosphate. The starch synthases, which catalyse the transfer of glucose from ADP-glucose to the non-reducing end of a growing α-1,4-glucan, are divided into two classes: the granule-bound starch synthases (GBSS) and the soluble starch synthases (SS). In both classes several isoforms have been described from many different plant species. The branching enzyme, which introduces branchpoints into the amyllopectin, can also occur in different isoforms.

Other enzymes present in plants, which also act on α-1,4-glucans, such as the starch phosphorylases, disproportionating enzyme and different starch hydrolases, might also be important for determining the starch structure and, therefore, its processibility. Many aspects of starch synthesis are not fully understood to date. Starch metabolism can be manipulated through genetic engineering, either by the ectopic expression of different heterologous genes or through the repression of the expression of endogenous genes using antisense RNA technology. This not only allows the functional analysis of starch biosynthetic proteins, but also the manipulation of starch structure in order to widen its industrial applications.

In this way many different potato lines have been generated, containing either different amounts of starch or which synthesise a structurally modified starch. These structural changes relate to the amyllose content, the phosphate content or the gelatinisation and gelation characteristics of the starch.

3 PRODUCTION OF HIGH OR LOW MOLECULAR WEIGHT FRUCTANS IN CROPS

In nature, polyfructosylsucrose (fructan) is produced by certain bacteria and some plant species. In bacteria two types of fructans can be found. Some strains produce a 2,6-linked polyfructosylsucrose (levan) whereas others accumulate the 2,1-linked type called inulin. Levans and inulins have a degree of polymerisation (DP) of more than 100,000 fructose moieties. Synthesis of inulin and levan based on sucrose as the substrate is each catalysed by only one enzyme, the so-called inulinsucrases and levansucrases, respectively.

Expression of levansucrase in transgenic potatoes led only to a minimal accumulation of levan, but these plants display a severe phenotype. The development of levansucrase expressing plants is retarded and the tuber yield decreased significantly. In contrast, expression of inulinsucrase from Stepnotococcus mutans did not effect growth, development or tuber yield of transgenic potatoes, but these plants accumulate inulin up to 30%. Due to the unfavourably high $K_m$ of the inulinsucrase for sucrose (80 mM sucrose) and the sucrose competing starch biosynthesis, the expression of the enzyme in a sucrose storing plant, such as sugar-beet or sugar-cane, might result in an economically acceptable accumulation of inulin. The usage of inulin is not restricted to its characteristics as a polymer but seems to be more important as a substrate for the production of pure fructose for food applications.

Inulin synthesised by plant enzymes is much smaller then bacterial polyfructose. In plants, two different enzymes are necessary to synthesise the polymer. In a first step catalysed by sucrose-fructosyl-transferase (SST) one fructosyl moiety is transferred from one sucrose molecule to another leading to the trisaccharide kestose and glucose. The fructanfructan-fructosyl-transferase (M) catalyse the transfer of fructosyl moieties from one fructan to another fructan molecule. This reaction leads to the accumulation of fructans with an average DP of 25–100, depending on the plant species.

Chicory is currently used for the commercial production of inulin from plants. It is clearly interesting to transfer the enzymes for fructan biosynthesis from a low-yield plant species to a high-yield crop like sugar-beet. To this end, cDNAs encoding SST and FFT from artichoke were isolated and characterised. Transient expression of both cDNAs in tobacco protoplasts results in the accumulation of polyfructose. Stable expression of both enzymes in sucrose-accumulating crops will hopefully lead to the production of a significant amount of polyfructose, which will allow the purification of fructans on a lower cost level.
4 PRODUCTION OF BIODEGRADABLE PLASTICS IN PLANTS

One of the most exciting potential uses of transgenic plants is the production of biodegradable plastics. Plastic produced in plants would be a renewable resource and theoretically could have a comparable cost to that of non-biodegradable plastics produced from oil. Given the enormous demand for plastic and the potential for this demand to increase as living standards rise in developing countries, the possibility of economic production of biodegradable plastic is a very exciting one that has inspired an increasing number of groups and companies to work in this area over the last decade. At prices competitive with oil-derived plastics (e.g. $1/kg) the potential US market for PHAs has been estimated to be in the range of 0.5–5 billion kg/year. Further, the potential for natural plastics to offer a range of properties not yet available adds extra impetus to research in this field. In this section we will outline the necessary steps required in order to produce PHA in transgenic plants and review the progress that has already been made towards this aim.

There are a range of genes from various microorganisms available for the metabolic engineering of plants. The initial strategy taken by the group of Chris Somerville, was to overexpress the genes from *A. eutrophus* in the cytosol of *Arabidopsis thaliana* to prove the principle of PHB production in plants. Poirier *et al.* transformed Arabidopsis with the reductase and synthase individually and then crossed lines homozygous for each transgene to produce lines expressing both genes. This strategy relied upon the presence of an endogenous activity in the cytosol capable of producing acetoacetyl-CoA from acetyl-CoA. Poirier *et al.* were able to identify PHB production in the transgenic lines, although at relatively low levels of 20–100 µg/g FW. Microscopic examination revealed that granules of PHB could be found in the cytosol, nucleus and vacuole of the lines expressing both *A. eutrophus* genes. Despite the relatively low production of PHB, the plants were badly affected by the expression of the transgenes. In particular, expression of the reductase alone led to up to a 45% reduction in biomass production over the first 22 days. Expression of the synthase alone did not lead to any adverse effects, but expression of both transgens together led to even more stunted growth than with the reductase alone. The authors therefore proposed that either the depletion of acetyl-CoA or the PHB granules themselves were toxic to the plants.

The same group then adopted a modified strategy of targeting the products of the transgenes to the plastid compartment. In this study they first produced three single transgenic lines expressing the thiolase, reductase and synthase from *A. eutrophus* targeted to the plastid. They then used sexual crossing to produce lines expressing all three genes. They were able to find 20–700 µg/g FW of PHB in 20 to 30 day old plants and up to 10 mg/g FW in leaves from senescent plants. These latter measurements corresponded to around 14% of the dry weight of the Arabidopsis leaves. PHB granules could be clearly distinguished in the chloroplasts by electron microscopy. Importantly, plants producing PHB in the plastid showed wild type growth and fertility.

The pioneering work of Professor Somerville’s group has clearly established the principle of PHA production in plants using the model system of Arabidopsis. The next challenge is to produce PHAs in crop plants. Various companies are currently following strategies to express the PHB synthetising genes in the plastids of oil-storing seeds, in the belief that this will be an excellent background to achieve high yields of PHB. Whether this turns out to be the case remains to be seen.

A further active research area will be the production of other types of PHA in plants. Here a better understanding of the pathways responsible for the production of various hydroxyacyl-CoAs with long side chains is required. It will be necessary to identify plants, tissues and compartments that can produce these compounds or to devise new methods for their production. Expression of the second class of PHA synthase, typified by those from pseudomonas, has not yet been reported in plants and the results of such studies are eagerly awaited.

Finally, more sophisticated uses may be found for PHA production in plants. John and Keller working at the company Agracetus have recently reported on the expression of the *A. eutrophus* reductase and synthase genes in cotton. These workers used the 35S promoter but focussed their investigations on the cotton fibres. They were able to find PHB in transgenic cotton fibres and this resulted in distinct changes in the properties of the fibres. They found that the fibres exhibited better insulating characteristics and had a higher heat capacity. Thus the introduction of PHAs into cotton fibres might improve and extend their properties.
In conclusion, the case for active research in the area of PHA production in plants is convincing. The principle of PHB production in a model plant, Arabidopsis has been shown, and it remains now for economic-scale production to be achieved in crop plants. The promise of the diversity of PHAs should also be followed in plants, as this could lead to a range of biodegradable plastics, sustainably produced, to suit many applications. Achievement of these aims will not be quick, but the rewards will be so great that they are worth pursuing.

REFERENCES

1. Munro, E. M., Cereal Foods World, 1994, 39, 552.