A plant binary vector with an antisense soybean UDP-glucose dehydrogenase gene

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ABSTRACT

Soybean seeds are a valuable food and feed source because of their high protein and oil content. Conventional breeding has had limited success in increasing both oil and protein. Reducing the amount of cell wall polysaccharide in soybean seed could allow higher protein and oil content, thus increasing their economic value. UDP-glucose dehydrogenase is a key enzyme in cell wall polysaccharide synthesis. To investigate the role of UDP-glucose dehydrogenase in seed development, a transgenic approach was initiated. A new binary vector has been constructed for soybean transformation that has the following features: *i*) the selectable marker hygromycin phosphotransferase (*hpt*); *ii*) placement of the selectable marker adjacent to the T-DNA left border and, *iii*) an antisense copy of the UDP-glucose dehydrogenase gene driven by the seed-specific vicilin promoter. This genetic construct was engineered to suppress the expression of UDP-glucose dehydrogenase in soybean seeds.

KEY WORDS: Genetic construct, soybean transformation, antisense technology, oil and protein content, binary vectors.

INTRODUCTION

Soybeans are an important component of both human and animal diets providing one of the most used sources of plant protein. The composition of mature soybean seed varies depending on variety and growing conditions, but in general averages 40% protein and 20% oil. Protein and oil content are negatively correlated, so that as oil increases, protein content is reduced. Of the remaining 40% seed dry weight, 35% is carbohydrate and 5% is ash. Pectin, hemicellulose and cellulose make up about 20% of the seed weight (Stombaugh et al., 2000). These cell wall polysaccharides have little or no nutritional or commercial value, and represent a major sink for assimilated carbon. Therefore, decreasing the proportion of these polysaccharides could potentially increase seed oil and protein content. Furthermore, conventional soybean breeding has had little success in simultaneously increasing protein and oil content.

UDP-glucose dehydrogenase (DH) is a major step in the synthesis of glucuronic acid, the main precursor for the monosaccharides incorporated into cell wall polysaccharides (Figure 1; Carpita and McCann, 2000). UDP-glucose dehydrogenase activity is found in many prokaryotic and eukaryotic tissues. This enzyme has been studied in a number of systems. It was first demonstrated in bovine liver (Strominger et al., 1954) and subsequently in pea seedlings (Strominger and Mapson, 1957).

Soybean UDP-glucose dehydrogenases have been characterized from nodules (Stewart and Copeland, 1998), seedlings (Tenhaken and Thulke, 1996) and developing seed (Litterer, 2000). Tenhaken and Thulke (1996) and Litterer (2000) cloned soybean cDNAs for the DH. The cDNA cloned by Litterer (2000) is expressed in developing soybean seed.

Both overexpression and suppression of a target gene have proven to be useful strategies for manipulating plant metabolism (Kinney, 1998). Expression of an antisense RNA molecule that is exactly complementary to the mRNA transcribed from the target gene results in formation of a RNA duplex that prevents translation, and thereby expression, of the target protein (Van der Krol et al., 1988). Our long-term goal is to investigate the role of DH in soybean seed cell wall polysaccharide biosynthesis. One approach towards this goal is to suppress the expression of DH during seed development. The objective of this study was to construct a binary vector for soybean transformation containing an antisense copy of DH that would suppress DH expression.

MATERIAL AND METHODS

In the course of soybean transformation experiments it is imperative to work with a transformation vector that contains selectable markers and unique restriction sites. Plant transformation vectors usually contain a selectable marker gene that allows selection of transformed tissue cultures and/or plants in the presence of a selective agent. Binary vectors, small T-DNA containing plasmids that can be propagated in both *Agrobacterium* and *E. coli*, are conveniently designed for insertion of a transgene of interest (Bevan, 1984). The transfer of T-DNA to plant cells during *Agrobacterium*-mediated transformation is initiated in a polar fashion at the right T-DNA border from binary and other T-DNA containing vectors (Zambryski, 1988). Therefore, the probability of selecting transformed plant cells that contain complete T-DNA insertions is increased by using constructs that contain a selectable marker gene located near the left border. Binary vectors with this configuration allow counter selection against partial T-DNA integration events (Becker et al., 1992).

The DH gene was cloned in antisense orientation into the binary vector pGPTV-HPT (Becker et al., 1992). The plant selectable marker gene *hpt* is driven by the nopaline synthase (nos) promoter and is adjacent to the left T-DNA border. The anti-DH gene was placed under control of the seed-specific vicilin promoter (Higgins et. al., 1988) oriented towards the right T-DNA border. The *hpt* selectable marker gene has been previously shown to provide efficient soybean transformation (Olhoft et al., 2003).

The cloning steps used in this construction were performed according to standard procedures (Sambrook and Russell, 2001). QIAgen kits for DNA purification, plasmid DNA extraction and gel extraction were used according to the manufacturer's instructions. After each step in the construction, the intermediate plasmids were subjected to restriction digestions to check for the inserted fragment in size and orientation. The T-DNA construction in the final plasmid was confirmed by sequencing. (Advanced Genetic Analysis Center, University of Minnesota).

The 1450 bp DH cDNA, which has a *Nco*I site 30 bp from the stop codon, was modified via PCR to add 5' *Sma*I and 3' *Sac*I sites by Dr. Deborah Samac (USDA-ARS, St. Paul, MN) and the fragment was cloned into pBS KS+ digested with *Sma*I and *Sac*I. From this plasmid, a *Sal*I/*Nco*I fragment was isolated and cloned into the same sites in pProEXHTa (LifeTechnologies). The vicilin promoter was isolated from plasmid pCW66 generously provided by T.J. Higgins, CSIRO, Canberra, Australia (Higgins et al., 1988) by double digestion with *Xba*I and *Nco*I to release a ca. 2.7 kb fragment. This fragment was ligated into the binary vector pGPTV-HPT with a stuffer sequence to preserve the *Sac*I site adjacent to the Nos 3' terminator sequence in the binary vector. The DH fragment was isolated from pPROEXHTa by *Nco*I/*Sac*I digestion and ligated into pGPTV-HPT digested with the same enzymes to produce the finished plasmid with the DH in antisense orientation relative to the vicilin promoter. A schematic diagram of the region between the right and the left T-DNA borders of pUMN1-HPT-DH is illustrated in Figure 2. The engineered plasmid is 15,489 bp.

After completion, pUMN1-HPT-DH was introduced into *Agrobacterium tumefaciens* strain EHA 105 via electroporation. Studies on soybean transformation with pUMN1-HPT-DH are now in progress. The transformation efficiency of the pUMN1-HPT-DH vector into soybean cotyledonary-node cells using an *Agrobacterium*-mediated T-DNA method (Olhoft et al., 2003) was similar to previous experiments that used different binary vectors. Southern blotting analysis on regenerated, fertile plants indicated the recovery of 21 independent transformation events. Future research will concentrate on the level and stability of suppression of DH activity in each transformed line,

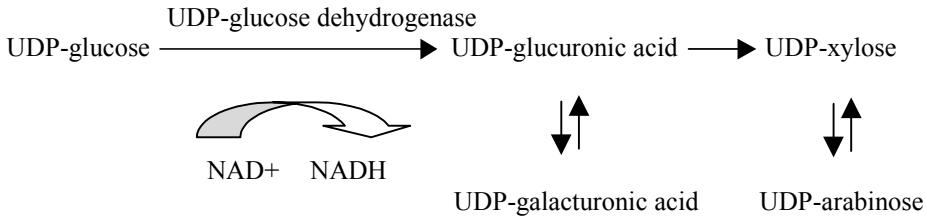

Figure 1. Biochemical pathway for the biosynthesis of precursor sugars of pectin and hemicellulose. Source: Carpita and McCann (2000) (adapted).

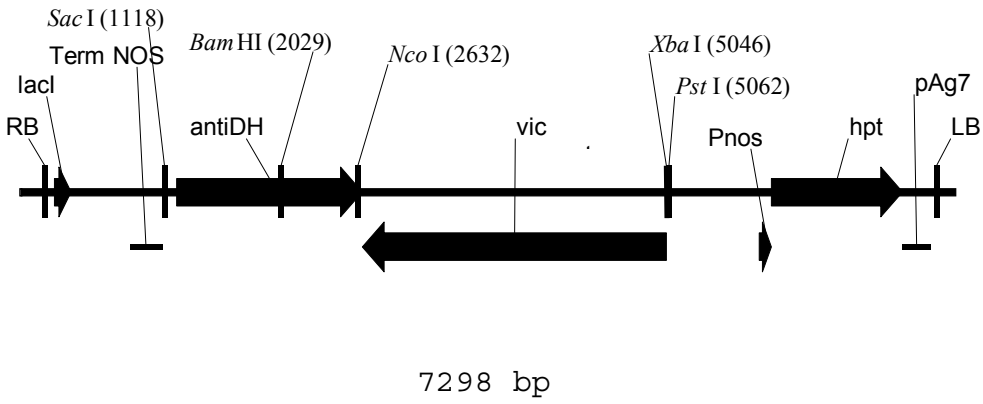

Figure 2. T-DNA structure of the pUMN1-HPT-DH binary plasmid.

and its impact on seed polysaccharide content and composition.

RESUMO

Vetor Binário com o gene anti-senso UDP-glucose Desidrogenase

As sementes de soja são uma importante fonte de alimentos em razão do seu elevado conteúdo de óleo e proteína. A redução da quantidade de fibras da parede celular nas sementes de soja pode contribuir para seu valor econômico. Um novo vetor binário foi construído com as seguintes características: *i*) marcador de seleção higromicina fosfotransferase (*hpt*); *ii*) marcador de seleção localizado próximo a margem esquerda do T-DNA, e *iii*) o gene anti-senso UDP-glucose desidrogenase sob a ação do promotor específico de sementes vicilina. Esta construção gênica foi obtida com o objetivo de reduzir a expressão do gene UDP-glucose desidrogenase em sementes de soja.

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