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Review

Tailor-made fructan synthesis in plants: A review

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ABSTRACT

Fructan, a fructose polymer, is produced by many bacteria and plants. Fructan is used as carbohydrate reserve, and in bacteria also as protective outside layer. Chicory is a commercial fructan producing crop. The disadvantage of this crop is its fructan breakdown before harvest. Studies using genetically modification showed that fructan biosynthesis is difficult to steer in chicory. Alternatives for production of tailor-made fructan, fructan with a desired polymer length and linkage type, are originally non-fructan-accumulating plants expressing introduced fructosyltransferase genes. The usage of bacterial fructosyltransferases hindered plant performance, whereas plant-derived fructan genes can successfully be used for this purpose. The polymer length distribution and the yield are dependent on the origin of the fructan genes and the availability of sucrose in the host. Limitations seen in chicory for the production of tailor-made fructan are lacking in putative new platform crops like sugar beet and sugarcane and rice.

Contents

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1. Introduction

Fructan is a polymer consisting of fructose units and a terminal glucose residue. Fructan occurs in a wide range of organisms; bacteria, some fungi and in about 15% of the flowering plants. Based on the linkage type fructan can be divided into three groups: (1) levan, with β(2-6) linked fructosyl units; this fructan type mainly occurs in bacteria (Dedonder, Neufeld, & Ginsburg, 1966) and monocotyledonous plants (where it is also called phlein) (Bonnett, Sims, Simpson, & Cairns, 1997), (2) inulin, a β(2-1) linear polymer that is found in dicotyledonous plants (Koops & Jonker, 1996), and (3) fructan neo-series, a mixed type of fructan found in Liliaceae (Pollock, 1986) in which β(2-1) chain elongation occurs on the C1 and the C6 positions of the glucose residue.

Bacteria use fructan as energy storage molecule (Burne, Chen, Wexler, Kuramitsu, & Bowen, 1996) and as a protective layer outside the cell. This fructan layer is used by plant pathogenic bacteria for blocking host–pathogen recognition and against bacteriostatic compounds released by collapsed plant cells (Kasapis, Morris, Gross, & Rudolph, 1994). Streptococci, present in the oral cavity, use the fructan layer as adhesive and as such fructan is important in the formation of dental plaque, which consists for a large part of levan-type fructan (Cote & Ahlgren, 1993). The biosynthesis of levan in bacteria is performed by a single enzyme, levansucrase (E.C. 2.4.1.10).

In plants, fructan serves as a reserve carbohydrate and is stored in stems, tubers or taproots. It has also been suggested that fructan protects the plant against drought and cold stress (Pilon-Smits et al., 1995; Pollock, 1986). The length of plant fructan varies from 10 to approximately 200 fructosyl units. This variation highly depends on the taxonomic diversity of fructan producing plant species. In contrast to bacteria, the biosynthesis of fructan in plants is catalysed by three different classes of enzymes: sucrose:sucrose 1-fructosyltransferase (EC 2.4.1.99) (1-SST), fructan:fructan 1-fructosyltransferase (EC2.4.1.100) (1-FFT) and fructan exohydrolase (EC.3.2.1.153) (1-FEH) (Edelman & Jefford, 1968). 1-SST primarily catalyses the synthesis of the trisaccharide 1-kestose, from two molecules of sucrose. In this reaction glucose is formed in equimolar amounts to 1-kestose. 1-FFT catalyses the transfer of fructosyl units from 1-kestose and any other fructan molecule onto 1-kestose and higher DP fructan molecules. 1-FFT increases the mean degree of polymerisation (mDP) when using 1-kestose, the shortest fructan, as a fructosyl donor because this reaction converts 1-kestose into sucrose, which does not count up as fructan molecule, and a fructosyl unit that is used to elongate a pre-existing fructan molecule. Under 1-kestose limiting conditions, for example when 1-SST activity is low, 1-FFT can also catalyse the transfer of fructosyl units from a fructan molecule onto sucrose (Van den Ende, De Roover, & Van Laere, 1996; Vergauwen, Van Laere, & Van den Ende, 2003), this so-called 'back transfer' reaction results in the decrease of the mDP. The third class of enzymes, 1-FEH, catalyses the degradation of inulin by hydrolysing terminal fructosyl units, which results in the formation of fructose and lower DP inulin (Van den Ende, Michiels, Van Wonterghem, Vergauwen, & Van Laere, 2000).

Plant fructan is used for a range of food and non-food applications (Sévenier, van Arkel, Hakkert, & Koops, 2006) depending on the degree of polymerisation (DP). Short chain inulin is used, for example, for the production of fructose syrup, mainly used for the sweetening of cold drinks. Long chain inulin (mDP $\geq$ 25) is used as fat replacer and foam stabiliser in food products. Long chain inulin is also starting material for the production of carboxymethylinulin, a scavenger of divalent cations in household detergents. The crop that is grown for the production of fructan on a commercial scale is chicory.

Chicory (*Cichorium intybus* L.) is a biennial taproot-bearing crop that is sown in spring and the taproots are harvested in autumn of the same year. Inulin is extracted from the taproot. At harvest, the mean inulin polymer length is 9–10 and the average yield is about 11,000 kg carbohydrate/ha (Wittouck et al., 2002).

One of the most important quality parameters of inulin is the polymer length. For certain applications, like fat replacer which require a minimum DP > 25, the raw inulin extracted from chicory is unsuitable and should be enriched for long molecules, which is a costly process.

Interestingly, it has been observed in chicory, that the polymer length earlier in the growing season is much higher than at harvest. The lower polymer length at harvest is caused by catalytic reactions of 1-FFT and 1-FEH, both enzymes responding to plant and environmental factors.

Plants that naturally accumulate fructan can also degrade the polymer in order to remobilize the stored carbon. This catalytic breakdown of fructan is a major drawback in the production of inulin as is also observed in the crop chicory. Several solutions for this problem of breakdown have been proposed in the past years, including attempts to generate new fructan accumulating plant species by genetic modification of crops that originally did not synthesize (and degrade) fructan.

An additional advantage of using non-fructan-accumulating plant species for the commercial production of fructan is that highly productive crop plants, having well-established husbandry and processing chain, can be chosen. Moreover, the introduction of fructan biosynthesis in non-fructan species renders new types and different sizes of fructan not yet present in natural fructan-producing plants: the tailor-made fructan.

In this paper an overview is given of the research on modification of fructan synthesis in fructan accumulating plants on one hand, and the research on introduction of fructan synthesis in non-fructan-accumulating plants on the other hand. As most of the work on modification of fructan synthesis in fructan accumulation plants is performed on chicory, an introduction to the inulin metabolism of chicory is given. A wide range of bacterial and plant fructosyltransferase genes has been introduced in many different plants varying from monocots, like rice, maize and sugar cane, to dicots, like potato, sugar beet and clover. The effect of the modification or introduction of fructan synthesis is valued on the resulting fructan yield, polymer length and altered linkage type of fructan.

2. Modification of fructan synthesis in fructan-accumulating plants

2.1. Fructan biosynthesis in chicory

Chicory is a biannual crop that is grown for the production of inulin, which is stored in the taproot. The taproot starts to thicken seven weeks after sowing, concomitant with the induced activity of the fructan synthesis enzymes 1-SST and 1-FFT. The activity of 1-SST increases rapidly until it reaches a maximum three weeks later (Druart et al., 2001). Ten weeks after sowing the activity decreases until the end of the growing season in November, when only 10% of the activity is left (Van den Ende, Mintiens, Speleers, Onuoha, & Van Laere, 1996). The activity of 1-FFT follows a different pattern, the activity increases slowly and stabilises after four weeks. The activity of 1-FFT remains stable during the rest of the growing season (Van den Ende, Michiels, De Roover, & Van Laere, 2002). As a result of the fructosyltransferase activities in the first months, mainly short inulin is formed. At nine weeks after sowing inulin molecules with a DP up to 25 are accumulated. The mDP reaches a maximum of about 16 in the beginning of September and then decreases as reported by Druart et al. (2001). This extent of decrease