Review

# Plant fructans: Recent advances in metabolism, evolution aspects and applications for human health

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**Abstract:** Fructan, a fructose polymer, is used as carbohydrate reserve in many plants. The nutritional and therapeutic benefits of fructans have attracted increasing interest by consumers and food industry. In the course of evolution, many plants have developed the ability of regulating plant frunctan metabolism genes to produce different structures and chain length fructans, which are strongly correlated with their survival in harsh environments. *De nevo* domestication of fructan-rich plants based on genome editing is a viable and promising approach to improve human dietary quality and reduce the risk of chronic disease. These advances will greatly facilitate breeding and production of tailor-made fructans as a healthy food ingredient from wild plants such as polygonati rhizoma. The purpose of this review is to broaden our knowledge on plant fructan biosynthesis, evolution and beneficial applications for human health.

**Keywords:** plant fructans; fructosyltransferase; metabolism; evolution aspects; functional foods

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## 1. Introduction

Plant fructans are fructose polymers derived from sucrose and stores in vacuoles [1, 2]. They often act as a long-term carbohydrate in the specialized organs of perennial plants, the taproot of chicory (*Cichorium intibus*), the rhizome of *Polygonatum cyrtonema*, the stem of agave species, the bulb of tulip (*Tulipa gesneriana*) and onion (*Allium cepa*) (Figure 1) [3-5]. As structurally flexible and high soluble sugars, plant fructans play a key role in plant regrowth after defoliation and sprout. In addition, fructans can associate with cell membranes to help plants survive in harsh environments such as chilling and dry climates during the long evolution [6-9].



**Figure 1. Presentative species containing different fructan structure.** Onion, *Allium cepa*; Cocksfood, *Dactylis glomerate*; Barley, *Hordeum vulgare*; Chicory, Cichorium intybus; Oat, Avena sativa; Timothy, Phleum pretense; Asparagus, Asparagus officinalis; Jerusalem artichoke, Helianthus tuberosus; Wheat, Triticum aestivum; Agave, Agave Americana; Ryegrass, Secale cereal; *Polygonatum cyrtonema*.

Due to the fructose-linked molecules, fructans are not digested in human stomach or small intestine, but can be fermented by bacteria in the large intestine, producing short-chain fatty acids (SCFAs) [10-12]. Therefore, fructans can efficiently regulate blood glucose and reduce the risk of obesity [13]. Fructans, also as popular prebiotics, help reduce the risk of cancer, promoting the probiotic activity of bifidobacterium and lactobacilli and the absorption of minerals in human [14]. Nowadays, fructans have become a popular functional food because of its great benefits for human health.

Wild plants rich in fructan have attracted great attention from crop breeders. Domestication of the rich variety of nutritious and functional plants is considered to have enormous potential in agricultural and food industry [15]. *Polygonatum cyrtonema* is a traditional wild plant with dual purposes as food and medicine for around 2000 years in China. Current research showed that rhizome of *P. cyrtonema* does not contain starch, but is rich in fructan which makes the plant highly suitable for domestication [9]. Traditionally, development of commercial crops is the result of long-term domestication including natural and human selection, which is a time-consuming and labor-intensive process [16-18]. The recently developed genome editing technology has become a robust approach for targeted gene editing in a wide array of plants in just a few generations [16]. Domestication of fructan-rich wild plants with traditional or genome editing methodology will be a novel approach for improving human dietary choices for prevention and control of chronic disease such as diabetes and cancer.

#### 2. Fructan structures and metabolism in plants

Plant fructan chain lengths range from three up to a few hundred fructose units, mainly including inulin, neo-inulin, levan, neo-levan, graminan structures (Figure 2) [7, 19, 20]. fructans are biosynthesized by fructosyltransferase (FT) including 1-SST, 1-FFT, 6-SST/6-SFT, and 6G-FFT, and are broken down by fructan exohydrolases (FEH) [20].



**Figure 2. Schematic representative structure of plant fructans.** Sucrose in yellow frame including red-glucose and green-fructose; fructosyltransferase in blue color, sucrose:sucrose 1-fructosyltransferase (1-SST), fructan:fructan1-fructosyltransferase (1-FFT), sucrose:fructan 6-fructosyltransferase (6-SFT), fructan:fructan 6Gfructosyltransferase (6G-FFT), fructan exohydrolase (FEH).

One of the simplest form of fructans is inulin, which consists of  $\beta(1-2)$ -linked fructose style synthesized by 1-SST and 1-FFT [21, 22]. 1-SST is responsible for the conversion of sucrose to 1-kestose. 1-FFT is able to use 1-kestose or other high polymerization fructans as fructose donors, and fructans or sucrose as fructose acceptors, which determines the length of inulin in plants. Representative species of inulin are chicory and *Jerusalesm artichoke* (Figure 1). Compared with inulin, neoinulin in Liliacease (e.g. onion and asparagus) has one more linked style  $\beta(2-6)$  with glucose of sucrose synthesized by 6G-FFT.

The levan structures include levan and neo-levan, both having the basic linear  $\beta$ (2-6)-linked fructose structure attached to sucrose. Levan fructans are synthesized by species-specific enzyme 6-SST/6-SFT which preferentially transfers the fructosyl unit of sucrose to a wide variety of acceptors such as 1-kestose, 6-kestose and 6G-kestotriose [23-25]. Fructan neo-levan are formed through transfer of fructose residues to the glucose moiety of sucrose to form a 6G-kestose catalyzed by 6G-FFT [26]. 6G-kestose can be further elongated to form neo-inulin and neo-levan type fructans by 1-FFT and 6-SFT, respectively [27, 28]. In plants, levan is commonly found in grasses, including *Dactilys glomerata* and *Phleum pratense*, whereas neo-levans are found in a few Poales order species (e.g., oat and ryegrass).

Graminan and agavin structures are the special types combining both inulin and levan, having both  $\beta(1-2)$  fructose and $\beta(2-6)$  fructose residues. Graminans are widely found in plant species belonging to Poales order, such as wheat and barley. Compared with graminans, agavin type fructan include more $\beta(2-1)$  fructose chains attached to fructose, mainly existing in Agave species.

Fructan exohydrolases (FEH) are known to cause fructan breakdown that may be specific for  $\beta(2-6)$  or  $\beta(2-1)$  linkages or act on both [20]. The 1-FEH is responsible for the breakdown of inulin-type fructans with only  $\beta(2-1)$  linkages, while the 6-FEH degrades levan-type fructans exhibiting  $\beta(2-6)$  linkages [32]. Fructans with  $\beta(2-1)$  and  $\beta(2-6)$  linkages can be hydrolyzed by the 1&6-FEH that exhibited a preferred activity for bifurcose [29, 30].

Although fructan metabolism enzymes are thought to be transcriptionally regulated, the transcription mechanism of the fructans accumulation and stress signaling is still unclear. Recently, a R2R3-MYB transcription factor, TaMYB13, has been characterized as a transcriptional activator with the DNA-binding motifs (DTTHGGT) of the fructan biosynthesis genes, revealing fructosyltransferase genes co-expression mechanism driven by transcription factor [31, 32]. Interestingly, another recently identified chicory R2R-MYB factor, CiMYB17, is able to activate both fructosyltransferase (1-SST, 1-FFT) and fructan exohydrolase (1-FEH1, 1-FEH2) genes, via binding to the DTTHGGT cis-elements [33]. In fructan degradation pathway, CiMYB5 and CiMYB3 have been identified to regulate the expression of 1-FEH genes in chicory [34]. However, CiMYB3 transcripts only showed similar co-induction with FEH genes in cold-treated hairy roots but the correlation with expression of 1-FEH

genes was less consistent during different stress exposures and hormone treatments, indicating these R2R3-MYB regulators are expected to cooperatively or antagonistically regulate the expression of 1-FEH genes in a stress-dependent manner.

In addition, Jin et al. discovered a mechanism model of starch and fructan synthesis in barley, whereby a dual-promotor gene ecodes two functionally distinct transcription factor, SUSIBA1 of 30 kDa and SUSIBA2 of 62 kDa [35]. SUSIBA1 serves as a repressor that directly suppresses the synthesis of fructan, whereas SUSIBA2 is an activator that promotes the synthesis of starch. Depending on different sucrose concentration, the SUSIBA2 expression is controlled by SUSIBA1/SUSIBA2 competitive binding on the SUSIBA2 promoter, generating an autoregulatory system for the coordinated synthesis of starch and fructan. Interestingly, abundant fructans were accumulated instead of starch in rhizome of *Polygonatum cyrtonema*, providing a new question as for how carbon allocation is regulated [9].

### 3. Evolutionary aspects of fructan synthesis

The reason for the vast variety of fructan structures and different lengths chains in plants is unknown. This might be associated with different physiological needs or the consequence of the different evolutionary origins of fructan biosynthesis.

The biochemical and molecular experiments showed fructosyltransferases evolved from invertases by relatively few mutational changes [36]. Cell wall and vacuole invertases are acid invertases, which are also called  $\beta$ -fructofuranosidases breaking disor oligosaccharides from fructose residues [37-39]. The acid invertases belong to the glycoside hydrolase family (GH) 32 [40]. Compared with acid invertases, the alkaline/neutral invertases (CINs) that lack glycosylated activity and N-terminal signal peptide, specifically hydrolyze sucrose and fall in the GH100 family [37, 40]. CINs are likely to act in a different mechanism from acid invertases.



Figure 3. Evolutionary and Phylogenetic Analysis of invertase genes in plant and algal species. The amino acid sequence of invertases that have been experimental verification was used as database sequences; Query sequence in Table 1 were selected by the criteria of Evalue<10<sup>-10</sup> using BLASTP. The sequences were aligned with MAFFT (https://mafft.cbrc.jp/alignment/software/) and the phylogenies constructed with the raxmlHPC-HYBRID-AVX (http://www.phylo.org/). Esiliculosus chromista was used as the outgroup (the species are shaded) and the root of the tree, and the Interactive Tree of Life resource (http://www.itol.embl.de) was used to draw the phylogenetic tree.

Sequence comparisons of plant invertases showed a common evolutionary origin for vacuolar and cell wall invertases [41]. It is also possible they originated from duplication of a common ancestral gene, which may have occurred before the divergence of monocots and dicots [42]. In addition, it was previously hypothesized that plant VINs evolved from CWINs [43]. Recently a new finding shows that higher plant CWINs evolved from an ancestor shared with VINs from lower plant species [36]. However, our phylogenetic analysis showed VINs evolved from CINs, whereas CINs are more closely related to CWINs (Table 1; Figure 3). Thus, we proposed the evolution model of invertases that CWINs, CINs, VINs evolved from lower to higher plants corresponding with the emergence of the plant organ cell wall, cytoplasm, and vacuole.

The phylogenetic tree also suggests that plant fructosyltransferases evolved from vacuolar invertases, whereas fructan exohydrolases are more closely related to cell-wall invertases [7]. Our results indicated ancestral genes of 1-SST and 6-SFT were Vc1-SST and Vc6-SFT in Chlorophyta, respectively. Fructosyltransferases and vacuolar invertases from monocots occur in clade separate from those of the dicots, indicating that plant fructan genes evolved independently after the divergence of monocotyledons and dicotyledons [44]. However, our results revealed fructosyltransferase in both monocots and dicots were from a common origin gene AtVIN in basal angiosperm *Amborellale trichopoda*. For the 6-SFT genes from monocots were more similar to other invertases from monocots than to the fructosyltransferases from dicots. The 1-SST and 1-FFT from the dicot *Jerusalem artichoke* are more similar to dicot invertases than to fructosyltransferases from monocots [45]. These reusits suggested that different functional enzymes were sometimes more closely clustered than those that act the same reaction but originate from different families [7].

These evolution advances provide new opinions of fructan metabolism evolution and several frontiers in elucidating carbon allocation by the predictive function of invertases.

#### 4. Fructans application by human

#### 4.1 Potential benefits in human health

Fructans have been recognized as a beneficial food ingredient that is a soluble fiber that can escape digestion in most of the human digest system e.g. stomach, small intestine, which are metabolized by the beneficial michrobiota in the large intestine and thereby produce the major products short-chain fatty acids (SCFAs) [19, 46]. These beneficial flora and small molecules improve potential therapeutic benefits in human liver, pancreas, intestine and kidney (Figure 4).



Figure 4. The health benefits of plant fructans.

Inulin-type fructans have been studied for several decades that promote health in diverse ways. As a fructose linked prebiotics, inulin is fermented by beneficial bacteria in the large intestine or colon, producing SCFAs (acetate, propionate, butyrate) that are rapidly absorbed in the colon to provide additional energy to the host and improve the functioning of gastrointestinal microflora i.e. lactobacilli and bifidobactiria [47, 48]. SCFAs also prevent the establishment of potentially pathogenic intestinal microbes in balancing the colonic microflora towards a healthier condition in human [49, 50]. In addition, during the fermentation by the intestinal flora, SCFAs result in improving the gut environment, stimulating the growth of colonic mucosa and thereby increase the absorption of minerals [13]. Inulin-type fructans prebiotics also increase lipid droplets number, reduce fat mass, lipopolysaccharide, triglycerides, cholesterol, and free fatty acids in liver, and it have been further demonstrated potential protective effects on acute liver injury [51-53]. Recently studies also found fructans reduce serum total & free p-cresyl sulfate in chronic kidney disease patients and improve kidney function [19, 54].

Non-starch polysaccharides are important components of dietary fiber and contribute to treatment for diabetes [55, 56]. Lots of studies have revealed that the consumption of high fiber diet can increase the bulkness leading to increased gastric

emptying time [19]. Additionally, they also slow down the glucose absorption due to which sugar is released more slowly in the body thus can decrease the postprandial insulin level. Fructan-type plant polysaccharide, is widely found in nature and also reduce postprandial blood glucose and insulin response to foods [57-59]. *Lactobacillus plantarum* 1058 (ATCC 8014) and IN supplements is reported to lower hyperglycemia, insulin resistance and hyperlipidemia; reduce oxidative stress and increase the insulin and leptin levels in the hypothalamus of T2DM rats [60, 61]. Therefore, the fructans may regulate blood glucose levels in dual way.

#### 4.2 Accelerating the domestication of fructan-rich crops

Currently crop breeders focus on wild plants or neglected crops that consist of a larger extent on environmental sustainability, low input, and high nutritional/functional value [15, 62]. The health-promoting properties of fructans are crop breeding aim. Therefore, domestication of these crops is viable and promising approach to meet human's healthy needs.

The traditional domestication of wild plants into commercially available crops has usually taken hundreds or even thousands of years, but recently developed genome editing technologies provide the efficient way to accelerate the domestication by editing the domesticate genes [16]. A clear accelerated de novo domesticated path by combining the advantages of wild allotetraploid rice genome sequencing and editing for creating novel crops were demonstrated. These promising techniques have become useful tools in the plant breeding toolbox when domesticating new crops.

Perennial plants typically have more-advanced root systems which not only allows them to be less reliant on fertilizer and water supply but also contribute to preservation of soil quality [62, 63]. Thus domestication of perennials are in the breeding pipeline. Polygonati rhizoma is a perennial plant with a long growth period, large-scale cultivation, natural polyploidization. In addition, Polygonati rhizoma does not contain starch, but is rich in non-starch polysaccharides and fructans. Therefore, it may be a promising species to become domesticated crops with high nutrition and functional food in the future.

#### 5. Conclusions and Future perspectives

In conclusion, fructan is a fructose-based polymer that has great nutritional and health potential for promoting human health, including prebiotics effects, reducing the risk of colon cancer, reducing the risk of obesity and diabetes and so forth. This review systematically summarized the fructan style, functional enzymes and evolution aspects in fructan metabolism. Additionally, we provide new opinions related the origin and evolution of invertases including CWINs, CINs, VINs and FT from lower to higher plants. These advances provide new opportunities to select fuctan-rich wild plants and improve the domestication using biotechnological approaches. A presentative wild species, *Polygonatum cyrtonema* has been attracting huge interest in China, which fulfils as nutritional and functional diet especially for diabetes and elderly. Domestication of these fructan-rich plants would promote the diversity of agriculture and fill the current gaps between crop production and utilization of crop nutritional and functional values.

## **Tables and figures**

 Table 1. Species for phylogenetic tree construction. Invertases in different plant and algal species.

**Figure 1. Presentative species containing fructans.** Onion, *Allium cepa*; Cocksfood, *Dactylis glomerate*; Barley, *Hordeum vulgare*; Chicory, Cichorium intybus; Oat, Avena sativa; Timothy, Phleum pretense; Asparagus, Asparagus officinalis; Jerusalem artichoke, Helianthus tuberosus; Wheat, Triticum aestivum; Agave, Agave Americana; Ryegrass, Secale cereal; *Polygonatum cyrtonema*.

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Figure 4. The health benefits of plant fructans.

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