Multifunctional fructans and raffinose family oligosaccharides

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Wolfram Weckwerth, University of 1-FFT, fructan:fructan 1-fructosyl transferase; 1-SST, sucrose:fructan 1-fructosyl transferase; 1-FFT, fructan:fructan 1-fructosyl transferase; 1-SST, sucrose:fructan 6-fructosyl transferase; 6G-FFT, fructan:fructan 6G-fructosyl transferase; 6-GFT, fructan:fructan 6-G-fructosyl transferase; GS, galactan:galactan galactosyl transferase; GGT, galactan:galactan galactosyl transferase; GHXX, family XX polymerization; FEH, fructan exohydrolase; Fru, fructose; FT, fructosyltransferases; CWI, cell wall invertase; DP, degree of polymerization; IN, invertases; ST, stachyose; StS, stachyose synthase; sucr, sucrose; ViS, vacuolar invertase.

Introduction

Sucrose (Suc; Glc1,2[Fru]) takes a central position in plant metabolism as the first free sugar formed during photosynthesis and the major transport compound to bring carbon skeletons from source to sink tissues (Koch, 2004). Suc is the substrate for the synthesis of different types of Suc-derived oligosaccharides (Keller and Pharr, 1996). Among those, fructans and raffinose family oligosaccharides (RFOs) are the most important two classes of water-soluble carbohydrates in plants. Recent progress is summarized on their metabolism (and regulation) and on their functions in plants and in food (probiotics, antioxidants). Interest has shifted from the classic inulin-type fructans to more complex fructans. Similarly, alternative RFOs were discovered next to the classic RFOs. Considerable progress has been made in the understanding of structure–function relationships among different kinds of plant fructan metabolizing enzymes. This helps to understand their evolution from (invertase) ancestors, and the evolution and role of so-called “defective invertases.” Both fructans and RFOs can act as reserve carbohydrates, membrane stabilizers and stress tolerance mediators. Fructan metabolism can also play a role in osmoregulation (e.g., flower opening) and source–sink relationships. Here, two novel emerging roles are highlighted.

First, fructans and RFOs may contribute to overall cellular reactive oxygen species (ROS) homeostasis by specific ROS scavenging processes in the vicinity of organellar membranes (e.g., vacuole, chloroplasts). Second, it is hypothesized that small fructans and RFOs act as phloem-mobile signaling compounds under stress. It is speculated that such underlying antioxidant and oligosaccharide signaling mechanisms contribute to disease prevention in plants as well as in animals and in humans.

Keywords: antioxidant, fructan, immunity, oligosaccharide, raffinose, signaling, stress, sucrose
unclear and require further experimental verification. Fructans are believed to accumulate in vacuoles (Wiemken et al., 1988) but it was proposed that, under stress, tonoplast-derived vesicles may transport fructans from the vacuole to the apoplastic (Livingston and Henson, 1998; Valluru et al., 2008).

The "classic" RFOs are soluble, non-reducing α(1,6) galactooligosaccharides (Gals). Gal consists of Suc, Galα1,6Galβ1,2Fru is the smallest RFO and ubiquitous in the plant kingdom (Keller and Pharr, 1996). Further elongation with Gal residues leads to the DP4 stachyose (Sta; Galα1,6Galα1,2Fru, verbascose (DFP), ajugose (DF), etc. Classic RFOs with a DP up to 15 have been found after cold treatment in Ayuza repans L. (Bachmann et al., 1994), a typical RFO accumulator belonging to the Lamioceae. While Raf and Sta occur in all plant parts in genuine RFO accumulators, the higher homologues are usually restricted to the storage organs. Often Sta is the quantitatively dominating carbohydrate in such storage organs (Kandler and Hopf, 1984). Raf and Sta are also important transport compounds in the orders Lamiales, Caryophyllales, Cornales, and in one family of the Celastrales (Zimmermann and Zargler, 1975). Haritatos et al., 1996; Hoffmann-Thoma et al., 1998; Targone et al., 2011, recently, research has been devoted to so-called "alternative" RFOs in plants. These novel plant Gal oligosaccharides did not derive much attention in the past. Among these, the Sta derivative manninotriose (Galα1,6Galβ1,2Fru1,Galα1,2Galβ1) was found to be the predominant carbohydrate in cold-induced early spring red deadhead (dos Santos et al., 2013), a unique fructan since this compound was never observed before in any RFO accumulator (dos Santos et al., 2013). Intriguingly, Sta does not occur within the Caryophyllaceae. Instead, Raf is elongated to the DP4 lychnose (Galα1,6Galα1,2Fru1,Galα1,2Gal) and the DP5 stellariose (Galα1,6Galα1,2Galα1,4,Galβ1,2Fru1,Gal) in cold-treated Stellaria media (Vanhaecke et al., 2006, 2008, 2010).

**METABOLISM AND ITS REGULATION**

Sucrose is not only needed as a substrate for fructan biosynthetic enzymes (termed fructosyltransfereases: FTs), organ-specific Suc thresholds trigger the expression of genes encoding FTs (Lu et al., 2002; Maleux and Van den Ende, 2007) and RFO biosynthesis genes (Nägele and Heyer, 2013). Similar to the induction of anthocyanins in Arabidopsis (a non-fructan accumulator), it is well-known that fructan synthesis is controlled by a Suc-specific pathway (Bolouri Moghaddam and Van den Ende, 2013a and references therein), which means that the same effects cannot be obtained by using a mixture of Glc and Fru. Calcium, protein kinases and phosphatases are also involved in this inductive process (Martinez-Noil et al., 2009, 2010). Recently, the transcription factor TaMIB13 was found to be an important player in the process leading to FT induction and fructan synthesis in wheat (Xue et al., 2011) but further research into this pathway is needed to fully understand where this transcription factor is situated in the pathway. Even less is known about the pathway leading to RFO synthesis. However, it seems that heat shock transcription factors (HSFs), C-repeat binding factor/drought response element binding factor 1 (CBF/DREB1) type transcription factors and WRKY type of transcription factors (Panikulangara et al., 2004; Ogawa et al., 2007; Wang et al., 2009). Recently, it was reported that target of rapamycin kinase complexes stimulate the pathway leading to RFO synthesis in Arabidopsis (Dohrendorf et al., 2013 and references therein).

Inulin-type fructans are biosynthesized from Suc by two FTs. First, 1-kestotriose is produced by the activity of a sacrosesucrose 1-fructosyl transferase (1-SST) which transfers a fructosyl residue from a donor to an acceptor Suc. Then, a fructan:fructan 1-fructosyl transferase (1-FFT) polymerizes 1-kestotriose into higher DP inulin-type fructans (Fediman and Jefford, 1968; Van Laere and Van den Ende, 2002). Sucrose:fructan 6-fructosyl transferases (6-SFTs) are able to introduce branching. They preferentially transfer a fructosyl group from Suc as a donor substrate to 1-kestotriose as acceptor substrate, producing a 18th-kestotetraose (also termed bifucrose), the smallest graminan-type of fructan with mixed-type of linkages. Bifucrose can be further elongated by 6-SFT and 1-FFT, leading to branched, higher DP graminan-type of fructans (Yoshida et al., 2007). However, some of these 6-SFT enzymes might use Suc and/or 6-kestotriose as preferential acceptors, producing levan-type fructans (Tamura et al., 2009). Such 6-SST6-SFT is also involved in fructan synthesis in Pachysandra terminalis, although this particular enzyme also shows extensive hydrolytic activities as well (Van den Ende et al., 2011a; Lammens et al., 2012), and it can be considered as a "premature" FT [preliminary fructosyl transferase (pFFT); see also below]. Finally, the enzyme fructan:fructan 6G-fructosyl transferase (6G-FFT) synthesizes 6G-kestotriose (neokestose) from 1-kestotriose as donor substrate and Suc as acceptor substrate. Further elongation by 1-FFT and 6-SFT leads to the formation of inulin- and levan neoseries, respectively (Vijn and Smeekens, 1999). Plants use an array of different fructan exohydrolases (FEHs) to degrade their fructans (Van den Ende et al., 2004; Yoshida et al., 2007; Zhang et al., 2008), including 1-FEHs [preferentially attacking β(2,1) Fru linkages], 6-FEHs [preferentially attacking β(2,6) Fru linkages] and 6β1-FEHs [attacking both types of linkages]. These enzymes remove, one by one, terminal Fru units from fructan chains. In contrast to invertases, FEHs cannot use Suc as a substrate. Instead, many FEHs are directly inhibited by Suc at the enzyme level (Verbaart et al., 2007), which represents one of the most important ways of regulation, next to the control of FEH gene expression at the transcriptional level (Van den Ende et al., 2002a). Remarkably, some of the apoplastic localized FEHs show an extreme specificity for single fructan kestotrioses, and these are termed kestotriose exohydrolases (Van den Ende et al., 2005), indicating that these forms might play a role in fructan signaling events (Van den Ende et al., 2004). It is known since long that FEHs also occur in non-fructan accumulators. However, they are probably better considered as "defective invertases" with possible [artificial] FEH side activities. The role of these proteins remained enigmatic for a very long period. However, a recent breakthrough paper (Le Roy et al., 2013) shows that Nin88, an apoplastic defective invertase from tobacco lacking FEH side activities, acts as indirect activator of active cell wall invertases (CWIs) which are crucial players in overall plant development, especially seed and fruit setting (Ruau et al., 2012). Although the exact underlying regulatory mechanisms require further research, data indicate that Nin88 interacts with cell walls in such a way that active CWIs bind to the cell wall in a more productive way (Le Roy et al., 2013). This fits
nicely within the emerging concept that dead enzymes are very common in all kingdoms of life and that many of them fulfill crucial biological roles, as reviewed in a recent Science paper (Leslie, 2013).

The first committed step in RFO biosynthesis is the production of galactinol (Galol) from myo-inositol and UDP-Gal, a reaction catalyzed by galactinol synthase (GolS; Keller and Pharr, 1996). Next, GolS acts as a donor to deliver Gal to Suc, creating Raf. This is catalyzed by raffinose synthase (RafS). Stachyose synthase uses Gol as donor and Raf as acceptor to synthesize Sta (Keller and Pharr, 1996). GolS, RafS, and StaS are believed to localize in the cytosol, although the RFOs they produce might also enter the vacuole and the chloroplasts (Nägele and Heyer, 2013). In some species, higher DP RFOs are produced by the action of galactan:galactosyl transferases (GGTs; Bachmann et al., 1994), using RFOs as donor and acceptor substrates. Although the exact origin of mannino-oligosaccharide type of RFO in red deadnettle is not known, it was suggested that this compound results from invertase (β-fructosidase) activity on Sta (dos Santos et al., 2013). Lycuminose synthase and stellariose synthase are the enzymes involved in the biosynthesis of lycuminose and stellariose (Vanhaecke et al., 2010). RFO catabolism involves the activity of acid and alkaline α-galactosidases which sequentially remove the terminal Gal residues (Keller and Pharr, 1996), while β-fructosidases may produce melibiose (Gal1,6Glc) from Raf and manno-oligosaccharide from Sta (dos Santos et al., 2013). The so-called seed inhibition proteins resemble the enzymes involved in RFO catabolism, but only a few forms have been functionally characterized (Peters et al., 2010). Similar to defective invertases, it can be speculated that some of these forms may represent catalytically inactive forms, acting as regulatory proteins. Some forms may be involved in the degradation of RFOs acting as cellular signals (see below).

ENZYMES: STRUCTURE–FUNCTION RELATIONSHIPS

The overall classification into families of carbohydrate active enzymes is based on amino acid sequence similarities (Cantarel et al., 2009). This classification (i) reflects the structural features of these enzymes better than their sole substrate specificity, (ii) helps to reveal the evolutionary relationship between these enzymes, and (iii) provides a convenient framework to understand mechanistic properties (Henrissat and Romeu, 1995).

Plant acid invertases (β-fructosidases), including vacuolar invertases (VIs) and CWIs, split Suc into Fru and GIC by hydrolysis of the glycosidic bond. FEHs hydrolyze a terminal Fru from a fructan chain, while FTs elongate a Suc or fructan molecule with an extra Fru moiety. Taken together, all these enzymes transfer a Fru unit either to water (hydrolysis), to Suc or fructan (Van den Ende et al., 2009). They only differ in their specificity for donor and acceptor substrates. Accordingly, the 3D structure determinations of a FEH from chicory (Verhaest et al., 2003), a CWI from Arabidopsis (Lammens et al., 2008) and a βFT from Pachysandra terminalis (Lammens et al., 2012) showed that all these enzymes (or proteins: defective invertases) have a common fold. Hence, they are grouped together with microbial β-fructosidases (degrading both Suc and fructans) in the family D2 of glycoside hydrolases (GH32). Family GH32 is combined with family GH68 in the clan GH-1. GH68 harbors bacterial invertases, levansucrases and inulosucrases. All these proteins consist of an N-terminal five-bladed β-propeller domain (GH32 and GH68) followed by a C-terminal domain formed by two β-sheets (only in GH32). The active site is present within the β-propeller domain and characterized by the presence of three highly conserved acidic groups (present in the WMDP), RDF, and EC-motifs. The Asp from the first motif is acting as nucleophile, the Asp from the second motif is believed to be a transition state stabilizer and the Glu residue from the EC motif acts as acid/base catalyst playing a crucial role in the catalytic mechanism (Van den Ende et al., 2009). Some sugars can bind as substrates or as inhibitors in the active site of plant GH32 members (Verhaest et al., 2007) and this depends on subtle amino acid variations in the active site area. Recent pKa calculations suggest that most GH-1 members show an acid-base catalyst that is not sufficiently protonated before ligand entrance, while the acid–base can be fully protonated when a substrate, but not an inhibitor, enters the catalytic pocket (Yuan et al., 2012).

Moreover, the conserved arginine in the RDP motif, rather than a previously proposed Tyr in the FYASK motif, is proposed to play a key role to increase the pKa of the acid–base catalyst (Yuan et al., 2012).

Intriguingly, defective invertases are never affected in their catalytic triad, but rather in a neighboring “Asp/Lys” or “Asp/Arg couple” (present in a flexible loop in the proximity of the acid/base catalyst) and in some Trp residues (Le Roy et al., 2007, 2013). These residues are essential to stabilize the Glc part of Suc in the active site of GH32 Suc splitting enzymes (CWINV, VI, 1-SST, 6-SFT; Van den Ende et al., 2009), and they are absent in enzymes that use fructans as donor substrates (FEH, 1-FFT, 6G-FFT). This was confirmed by site directed mutagenesis experiments on invertase, defective invertase, FEH and 6G-FFT (Le Roy et al., 2007, 2008, 2013; Lassere et al., 2009). However, the presence of an Asp/Lys or Asp/Arg is not sufficient; this couple needs to be in the right 3D configuration as well (Schroeven et al., 2009). The recent 3D structure of Pachysandra terminalis with its acceptor substrate t-kestotriose strongly suggested that the couple (Asp/Gln in this case) plays a prominent role in acceptor substrate specificity as well (Lammens et al., 2012).

All RFO metabolizing enzymes discussed in the previous section, with the exception of GolS, belong to GH27 and GH36 in clan D. The acid α-galactosidases and GGTs are grouped into GH32, where some 3D structures have been determined, including the acid α-galactosidase from rice (Fujimoto et al., 2003). Their active sites are well-conserved and formed by residues in the loops at the ends of the β-strands in a β(8)-barrel. Two Asp residues are required for catalysis, which are positioned on opposite sides of the labile glycosidic bond (Fujimoto et al., 2003). RafS, StaS, and alkaline α-galactosidases belong to the related GH68, but no structural information is yet available on plant members within this family (Vanhaecke, 2010), although a few microbial structures became available (Fredslund et al., 2011; Mecerem et al., 2012). To our knowledge, no in depth structure–function research has been performed toward donor and acceptor substrate specificities within plant members of GH27 and GH36. Clearly, such studies would be

http://www.cazy.org/
very informative as well. Such insights greatly contribute to rational enzyme design contributing to the production of tailor-made fructans and RFOs.

**EVOLUTION**

Within GH32, it became clear that plant FTs evolved from VIs (Wei and Chatterton, 2001; Altenbach et al., 2009), contributing to the observed diversity in fructan accumulators in the plant kingdom (Figure 1). Two types of VIs (I and II, Van den Ende et al., 2002b) can be discerned in plants and for a long time it was assumed that all plant FTs evolved from (different forms of) type II VIs. This occurred at least three times: (i) in the Asterales (inulin-type of fructans, e.g., chicory), (ii) in the Poales with further distinction between cool-season grasses (mainly levan and neokestose-derived fructans, e.g., ryegrass) and cereals (predominantly graminan-type fructans, e.g., wheat and barley) in the Poaceae and (iii) in the Asparagales further splitting into the Allioideae (e.g., onion) and Agavoideae (e.g., Agave) subfamilies that also mainly accumulate neokestose-based fructans (Figure 1). However, this view was changed by the unexpected discovery of both levan- and graminan-type fructans in the basal eudicot *Pachysandra terminalis* species, containing a pFT that, surprisingly, evolved from a type I VI (Figure 2) and not from a type II VI as observed for all other FTs (Figure 2). This further confirmed the polyphyletic origin of fructan biosynthesis (Altenbach et al., 2009; Van den Ende et al., 2011a) and suggests that the capacity for fructan biosynthesis arose at least four times during the plant diversification process (Figure 1). Such polyphyletic origin did not likely occur within GH27 and GH36, although more sequences should be generated to reach this conclusion (Vanhaecke, 2010). By combining alignments, 3D structure information and phylogenetic analyses (Schroeven et al., 2008; Altenbach et al., 2009; Lasseur et al., 2011; Lammens et al., 2012), the current view within GH32 is that an ancestral VI duplicated in two VI types (I and II, Figure 2) before the separation of monocots and dicots (Wei and Chatterton, 2001). Most probably, monocot and dicot type II VIs were than recruited to create preliminary 1-SSTs and 6-SFTs that later specialized into genuine 1-SSTs and 6-SFTs (Figure 2).
in the "WMNDPNG" and "W(A/G)W" motifs are believed to play a key role in such processes (Schroeven et al., 2008, 2009; Altenbach et al., 2009). In evolutionary terms, it seems reasonable to assume that, in monocots as well as in dicots, 1-FFT and 6G-FFT evolved later, likely from (premature) 1-SST precursors (Figure 2). For instance, in wheat, the identity between Ta1-SST and Ta1-FFT is much higher (84%) than between Ta1-SST and Ta6 (67%) and between Ta1-FFT and Ta6 (66%), strongly suggesting that Ta1-FFT evolved from Ta1-SST (Figure 2; Schroeven et al., 2009). A similar reasoning led to the hypothesis that the Lolium perenne Lp6<sup>66</sup>-FFT evolved from a (premature) Lp1-SST (Figure 2; Lassueur et al., 2009). In the same way, it can be speculated that the chlorella C31-FFT evolved from a (premature) C31-SST (Figure 2; Schroeven et al., 2009). Within the basal eudicots, a type I VI developed into a pFT in Pachysandra terminalis (Figure 2) and this is considered as a rather "recent" evolutionary event (Van den Ende et al., 2011a). On the contrary, defective invertases and FEHs evolved from CWIs within GH32 (Le Roy et al., 2007, 2013). It can be speculated that the loss or alteration of the above-mentioned "couple" is an early evolutionary event that led to the formation of defective invertases with cell wall localization and a high isoelectric point (pI) for interaction with the cell wall. To further develop genuine FEHs in fructan plants, it can be further hypothesized that precursor defective invertases retrieved (i) a vacuolar targeting signal for sorting to the central vacuole, (ii) a low pI typical for vacuolar proteins, (iii) amino acid alterations that helped stabilization of higher DP fructans as donor substrates (Le Roy et al., 2008).

CLASSIC FUNCTIONS OF FRUCTANS AND RFOs

The most widely accepted function of fructans is their role as a storage carbohydrate. Dictos typically store inulin-type fructans in underground reserve organs (roots, tubers) (Van Laere and Van den Ende, 2002) while monocots typically store fructans on a shorter term basis in above ground parts of the plant (Pollock and Cairns, 1991; Sweinski, 2012). To the best of our knowledge, fructans are the only natural type of polysaccharides that accumulate in plant vacuoles. Fructans can accumulate to 20% on fresh weight basis and even up to 78% on a dry weight basis in some organs (Wiemken et al., 1999). Trying to solubilize such levels in vitro invariably leads to fructan precipitation, suggesting that fructans in vivo should be organized in a special way to keep them in a (semi)-soluble condition in the vacuole (Van den Ende, 1996). It is clear that starch is the most widespread reserve carbohydrate in the plant kingdom. On the one hand, insoluble starch granules represent a very elegant way of storing huge amounts of carbon in a very small volume. On the other hand, excessive amounts of water-insoluble starch would be physically destructive to the chloroplast, its site of synthesis and storage in leaves. Therefore, fructans may have some advantages as compared to starch. One of the arguments in favor of using fructans could be the fact that starch biosynthesis dramatically decreases when the temperature drops below 10°C, whereas fructan biosynthesis is much less sensitive to low temperatures (Pollock, 1986). Another difference between starch and fructans might include the speed of its breakdown and carbon remobilization. While a large array of different enzymes (dikinases, phosphatases, starch hydrolyases) are necessary to release small sugars from a starch granule (Stitt and Zeeman, 2012), water-soluble fructans are expected to be degraded much quicker by the action of FEHs as a single enzyme type. In grasses fructans are mainly stored in the leaf bases and used for regrowth after defoliation (Morvan-Bertrand et al., 2001). In cereals, fructans temporarily accumulate in stems and early in seed development (Van den Ende et al., 2003; Van den Ende et al., 2011b; Joudi et al., 2012) as well as in reproductive organs (Ji et al., 2010). Contrary to the situation in dicots, where growth and fructan accumulation are usually separated in time, monocots are able to combine these processes. It could be argued that the activity of Suc splitting enzymes 1-SST and 6-SFT contribute to control and maintain sink strength and carbohydrate supplies (Ji et al., 2010), but then the obvious question can be raised why this is not simply accomplished by increasing the activity of invertases? This indicates that the accumulation of fructans as such should somehow be beneficial (see also below), especially under stress.

Fructans can also play a role during flower opening. Fructan contents are high in closed petals of Campanula rapunculus and Hemerocallis while no fructan is present anymore in petals while no fructan is present anymore in petals (Bieleski, 1993; Vergauwen et al., 2000). FEHs quickly release massive amounts of Fru, lowering the osmotic potential and contributing to water inflow and flower opening. Fructans appear to have additional functions in drought, salt, and freezing tolerance of plants (Valluru and Van den Ende, 2008; Livingtson et al., 2009). This is further supported by the fact that fructan-accumulating plants are especially abundant in temperate and arid climate zones with seasonal frost or drought periods, and are almost absent in tropical regions (Hendry, 1993).
Van den Ende Fructans and RFOs

In fructan species, fructan accumulation can be induced under drought (De Roover et al., 2008) and cold (Livingston and Hen-son, 1998; Yoshida et al., 2007). More direct evidence comes from the observation that fructan-accumulating transgenic plants show enhanced stress tolerance (Pilon-Smits et al., 1995, 1999; Konstantinova et al., 2002; Li et al., 2007; Kawakami et al., 2008; Bie et al., 2012). Transgenic perennial ryegrass expressing wheat 1-SST or 6-SFT genes accumulate more fructans and acquired higher tolerance for freezing at the cellular level (Hisano et al., 2004). Therefore, it would be interesting to introduce FT genes in a num-

ber of food and biomass crops, to make them more tolerant to abiotic stresses.

Next to fructans, RFOs are also used as "storage carbohydrates," arbitrarily defined as those which occur at more than 1% of the dry weight of a given tissue. So, despite the fact that most plants synthesize RFOs (at least Raf) to some extent at some stage of their development, only some plants accumulate large amounts of them (Kandler and Hopf, 1984; Keller and Pharr, 1996). These RFO accumulators store RFOs in concentrations up to 25–80% of their dry weight in specialized storage organs such as tubers (e.g., Stachys sieboldii), seeds (e.g., soybean, lentil, chickpea), or in photo-
synthesizing leaves (e.g., Ajuga reptans; Bachmann et al., 1994; Tahir et al., 2012). Similar to fructans, and in contrast to starch, RFOs are osmotically flexible as their DP may easily change and so has the osmotic pressure. Species that use Suc as reserve carbohydrate (sugar beet, sugar cane) can only double the osmotic pressure upon hydrolysis (Gilbert et al., 1997). Finally, RFOs are phloem-mobile, and are readily available for carbon translocation when required. This feature is less clear for fructans, since phloem mobility has only been documented in a single fructan accumulator (see below).

Typically, strong RFO and fructan accumulation do not occur together in a single plant species, suggesting that RFOs and fructans might fulfill similar (or partially overlapping) physiological functions. To better understand subtle differences in their physi-

ological functions, it would be interesting to seek for plants that are capable to store high levels of both RFOs and fructans. Similar to the introduction of FIs in non-fructan accumulators, the over-

expression of GolS in Arabidopsis thaliana resulted in plants with increased Raf levels and increased stress tolerance (Taji et al., 2002; Nishizawa et al., 2008). This suggests that the presence of increased levels of fructans or RFOs (in plants that normally contain very low or undetectable levels of such components) helps plants to survive adverse climatic conditions.

MEMBRANE STABILIZATION AND ANTIOXIDANT PROPERTIES

What could be the underlying mechanisms to explain such increased stress tolerance(s)? Since membranes (and critical mem-
brane proteins) are one of the primary targets of freezing and desiccation injury in cells (Oliver et al., 2000), membrane prote-
inve effects have been dedicated to fructans as well as to RFOs. In vitro experiments provided evidence for this ability, demonstrating that both fructans and RFOs contribute to enhanced membrane stability during freezing and cellular dehydration by deep inser-
tion between the headgroups of lipids, both in mono- and bilayers (Demel et al., 1998; Verryken et al., 2001; Hincha et al., 2002, 2003; Valleru and Van den Ende, 2008; Valleru et al., 2008). As such, they are also well-positioned to scavenge hydroxyl radicals (OH) which might originate from nonphotosynthetic Class III perox-

idase activities (Passardi et al., 2004; Van den Ende and Valluru, 2009). Among the biologically relevant reactive oxygen species (ROS: H2O2, O2− and OH), hydroxyl radicals are the most reactive and dangerous species (Krumen et al., 2013). The OH is known to react with almost all biomolecules at rates as those occurring in diffusion-controlled reactions (Hernandez-Marin and Martinez, 2012). As a consequence there are no enzymatic systems known to neutralize them in any living beings (Gechev et al., 2008). The in vitro OH scavenging activity of Raf and fructans has recently been confirmed (Stoyanova et al., 2011; Peshev et al., 2013) and compared to an array of phenolic compounds, well-known super-

ior antioxidants (Peshev et al., 2013). Based on these findings, a hypothetical model has been proposed explaining how vacuolar fructans and phenolic compounds may act in a synergetic way to contribute to vacuolar antioxidant mechanisms in vivo, and to overall cellular homeostasis (Peshev et al., 2013). While fructans are obvious candidates for nonphotosynthetic stabilization and protection, RFOs (Raf in cold-induced Arabidopsis leaves) that are synthesized in the cytosol are candidates to protect the plasma membrane. However, this seems not to be the target membrane in Arabidopsis (Nägele and Heyer, 2013). Instead, it was demonstrated that Raf specifically acts to protect the photosystems located in the thy-
lakoid membranes of plastids from damage during freeze thaw cycles (Knaupp et al., 2011). It was recently demonstrated that Raf can be imported in chloroplasts (Schneider and Keller, 2009) and therefore it could function as a cryoprotectant. As explained above for fructans or other osmolytes, it can be speculated that the OH scavenging capacity of Raf counteracts membrane and protein damage, contributing to thylakoid membrane stability and chloro-

plast integrity under stress (Dolchinkova et al., 2013). Likewise, targeting the synthesis of mannitol, another well-known OH scav-

enger (Stoyanova et al., 2011), to chloroplasts resulted in increased resistance to oxidative stress (Ohn et al., 1997a,b). Similar to what is observed in GolS overexpressors with their increased Raf levels (Nishizawa et al., 2008).

SIGNALLING?

Nowadays, Glc, Fru, and Suc-specific signaling pathways have been elucidated in plants (Rolland et al., 2006; Cho and Ioo, 2011; Li et al., 2011), already suggesting that a signaling role for other types of small endogenous sugars should not be simply neglected. It seems that (a) Suc-specific signaling pathway(s) contributes to plant defense responses (Bolouri Moghaddam and Van den Ende, 2013a,b). Increased Suc levels typically lead to increased levels of fructans, RFOs and/or anthocyanins (Teng et al., 2005; Martinez-Noel et al., 2009, 2010; Nägele and Heyer, 2013), perhaps controlled by (a single) Suc-specific signaling pathway(s) (Bolouri Moghaddam and Van den Ende, 2013a).

Gol and Raf are now recognized as signaling molecules during biotic stress responses (Kim et al., 2008) and a similar role during abiotic stress responses has been suggested for RFOs (Valleru and Van den Ende, 2011; Eyles et al., 2013) and for fructans (Van den Ende et al., 2004). This led to the hypothesis that both RFOs and small fructans might act as endogenous, phloem-mobile stress signals. Indeed, small fructans have been detected in the phloem
sap of Agave (Wang and Nobel, 1998) and it was reported that the fructan 6-kestotriose is phloem-mobile when it is produced by yeast invertase expressed in companion cells (Zetter et al., 2004). According to this view, the small fructans 1-kestotriose and its derivative isulobiose have been recently detected at very low levels in Arabidopsis, widely known as a strict non-fructan accumulator. What could be the origin of these small fructans in healthy Arabidopsis tissues? The most straightforward explanation is that these fructans are produced by the activities of VIs (AVII and AVII2), since Arabidopsis is lacking genuine FIs. Arabidopsis VIs were isolated before and found to contain considerable FT activities. Possibly, such signaling events form the basis of the so-called “sugar-based resistance” or “sweet immunity” concept (Gomez-Virto et al., 2007; Bolouri Moghaddam and Van den Ende, 2012, 2013b) in plants, but perhaps also in animals (see below).

UNIVERSAL IMMUNOSTIMULATORS?

Interest in fructans and RFOs increased during the last decade due to their health-promoting effects, selectively stimulating beneficial bacteria, acting as prebiotics (Booij et al., 2006; Urias-Silvas et al., 2008; Vanhaecke et al., 2012). Moreover, they are increasingly being recognized as important immunostimulators in animals and humans (Hettenje et al., 2005; Steifert and Watzl, 2007; Vos, 2008; Delgado et al., 2012; Lee et al., 2012) and as prebiotics (Ma et al., 2012). Taken all together, it can be speculated that these oligosaccharides may be involved in universal antioxidant and immunostimulatory mechanisms in plants, animals, humans, and perhaps in all eukaryotic organisms, but this requires further investigations. Needless to say, understanding the underlying mechanisms could greatly contribute to disease prevention strategies, both in plants and in mammals (Van den Ende et al., 2012; Bolouri Moghaddam and Van den Ende, 2012, 2013b; Di Bartolomeo et al., 2013).

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REFERENCES

Ahmedabadi, D., Badino-Pitara, E., Olbora, C., Rolle, T., Wiemken, A., and Rinsema, T. (2009). An arabidopsis subcellular fraction mining glycosyl transfer enzymes from mutant analysis of a plant vacuolar invertase and a fructan synthase. Plant Mol. Biol. 69, 47–56. doi:10.1007/s11103-008-8946-7

Bachmann, M., Mättig, P., and Keller, E. (1994). Metabolism of the radii-folia family oligosaccharides in leaves of Agave tequilana L. Cold acclimation, translocation and sink to source transition – discovery of a new elongation enzyme. Plant Physiol. 103, 1335–1345. doi:10.1104/pp.103.4.1335

Bielicki, R. L. (1995). Fructan hydrolase drives petal expansion in the ephemeral daylily flower. Plant Physiol. 103, 213–219

Bue, X., Wang, K., Shi, M., Du, L., Zhang, S., Li, J., et al. (2012). Combinatorial transformation of these wheat genes encoding fructan biosynthesis enzymes confers increased fructan content and tolerance to abiotic stresses in tobacco. Plant Cell Rep. 31, 2219–2226. doi:10.1007/s00299-012-1332-y

Bolouri Moghaddam, M., and Van den Ende, W. (2012). Sugars and plant innate immunity. J. Exp. Bot. 63, 1989–1998. doi:10.1093/jxb/erq317

Bolouri Moghaddam, M., and Van den Ende, W. (2013a). Sugar signaling in Arabidopsis thaliana. New Phytol. 196, 683–700. doi:10.1111/nph.12191

Bolouri Moghaddam, M., and Van den Ende, W. (2013b). Sweet immunity in the plant circadian regulatory network. J. Exp. Bot. 64, 1439–1449. doi:10.1093/jxb/ert169

Castillejo, B. L., Goñi, B., Gómez-López, V., Lombard, V., and Hernisot, B. (2009). The Cellobiose-Derived Enzymes database (CAZy): an expert resource for glycanomes. Nucleic Acids Res. 37, D233–D238. doi:10.1093/nar/gkn803

Castillejo, B. L., Zuazo, R., Cansado, E., Bautista, C., Bautista, E., Cacho, C., et al. (2011). Cellobiose/oligosaccharides of cereal wheat grain can be used as a potential feedstock for ethanol production. Bioresour. Technol. 102, 1617(11)80397-4

Cho, Y. H., and Yoo, S. D. (2011). Signaling role of fructan multidigested by FIS/DGBP in Arabidopsis thaliana. J. Genet. Genom. 38, 261–265. doi:10.1007/s12041-010-0028-8

Di Bartolomeo, F., Startek, J., and Kikkert, D., and van den Ende, W. (2008). Drought induces fructan synthesis and 1-5-5′-O-methyl fructofuranosyltransferases in roots and leaves of Celeriac cultivars (Celeri- crum carvifolium L.). J. Plant Physiol. 165, 552–556. doi:10.1016/S0176-1617(07)00397-4

L. Kohn, R., and Ramachandran, M., and Van den Ende, W. (2003). Tahir et al. (2009). Arabidopsis fructan synthase on (Solanum gilo) leaves. J. Photochem. Photobiol. B. 97, 206–217. doi:10.1016/j.jpbb.2009.09.012

Emberson, R. K., and R. A., and Sonnerat, M., and Sormani, R., et al. (2013). Sugar synthase and FIS/DGBP in Arabidopsis thaliana. J. Genet. Genom. 38, 261–265. doi:10.1007/s12041-010-0028-8

Di Bartolomeo, F., Startek, J., and Kikkert, D., and van den Ende, W. (2008). Drought induces fructan synthesis and 1-5-5′-O-methyl fructofuranosyltransferases in roots and leaves of Celeriac cultivars (Celeriac carvifolium L.). J. Plant Physiol. 165, 552–556. doi:10.1016/S0176-1617(07)00397-4

Dembir, R. A., Dorrer, E., Eshkamp, M. J. M., Smolensk, J. C. M., and de Kruijff, B. (1998). Fructans interact strongly with model membranes. Biochim. Biophys. Acta 1357, 36–42. doi:10.1016/S0005-2736(98)00280-2

De Rovers, L., Van den Ende, W., Van Laere, A., and Van den Ende, W. (2000). Drought induces fructan synthesis and 1-5-5′-O-methyl raffinose synthetase in roots and leaves of Celeriac cultivars (Celeriac carvifolium L.). J. Plant Physiol. 158, 109–114. doi:10.1016/S0176-1617(00)00305-3

Di Bartolomeo, F., Startek, J., and Kikkert, D., and van den Ende, W. (2008). Drought induces fructan synthesis and 1-5-5′-O-methyl fructofuranosyltransferases in roots and leaves of Celeriac cultivars (Celeriac carvifolium L.). J. Plant Physiol. 165, 552–556. doi:10.1016/S0176-1617(07)00397-4

Dembir, R. A., Dorrer, E., Eshkamp, M. J. M., Smolensk, J. C. M., and de Kruijff, B. (1998). Fructans interact strongly with model membranes. Biochim. Biophys. Acta 1357, 36–42. doi:10.1016/S0005-2736(98)00280-2

De Rovers, L., Van den Ende, W., Van Laere, A., and Van den Ende, W. (2000). Drought induces fructan synthesis and 1-5-5′-O-methyl fructofuranosyltransferas
The mechanism of fructan metabolism in higher plants as exemplified in Helianthus tuberosus. New Phytol. 67, 517–531. doi: 10.1111/j.1469-8137.1966.tb05480.x

Bushell, R., Belobrajdic, D. P., Le Mogne, J., Jenkins, C. L. D., Lewis, D., Sessions, L. C., Bird, A. R. (2011). Chain length of cereal fructans isolated from aleurone cell walls is a major carbohydrate in red dead-nettle (Lamium purpureum, Lamiales). Ann. Bot. 108, 363–380. doi: 10.1093/aob/mcr101

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Phytobiol. B 119, 23–30. doi: 10.1016/j.phytobiobio.2012.12.004

Jenkins, C. L. D., Lewis, D., Bird, A. R., Sessions, L. C., Bird, A. R. (2011). Chain length of cereal fructans isolated from aleurone cell walls is a major carbohydrate in red dead-nettle (Lamium purpureum, Lamiales). Ann. Bot. 110, 363–380. doi: 10.1093/aob/mcr101

Hoentjen, F., Welling, G. W., Harmening, D. M., Keller, F., Weller, A. (1996). Root-zone salinity alters raffinose oligosaccharide metabolism in higher plants. J. Exp. Bot. 47, 581–589. doi: 10.1093/jxb/47.343.581

Hendry, G. A. F. (1985). Evolutionary origins and natural functions of fructans—A dimensional, biogeographic, and mechanistic appraisal. New Phytol. 105, 3–14. doi: 10.1111/j.1469-8137.1985.tb01622.x

Hincha, D. K., Zuther, E., and Heyer, A. G. (2002). "Metabolism of carbohydrates in sinks and sources: glycolytic-sucrose-oligosaccharides," in Photosynthetic CO2 Distribution in Plants and Crops. Source-Sink Relationships, ed. K. Zamoski and A. A. Schaffer (New York: VCH Publishers), 115–132.

Kawashima, A., Sato, Y., and Yoshida, M. (2008). Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. J. Exp. Bot. 59, 803–814. doi: 10.1093/jxb/eru137

Keller, F., and Flute, D. M. (1994). "Metabolism of carbohydrates in sinks and sources: glycolytic-sucrose-oligosaccharides," in Photosynthetic CO2 Distribution in Plants and Crops. Source-Sink Relationships, ed. K. Zamoski and A. A. Schaffer (New York: VCH Publishers), 157–174.

Krauss, R., Podder, D., Vangronsveld, J., Van den Ende, W., and Corpuz, A. (2013). Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. Plant Cell Environ. 36, 204–215. doi: 10.1111/pce.12061

Kreuz, M., Kim, S. M., Kang, E. Y., Im, Y. J., Hwangbo, H., Kim, Y. C., et al. (2008). Galactinol is a signaling component of the induced systemic resistance caused by Pseudomonas chlororaphis O6 root colonization. J. Mol. Biol. 381, 216–226. doi: 10.1016/j.jmb.2008.03.004

Komatsu, M., Misawa, K. B., Nedbal, L., Knaupp, M., and Romeu, A. (1995). Comparison of fructan dynamics and substrate binding. J. Biol. Chem. 270, 20313–20318. doi: 10.1074/jbc.270.34.20313

Kothe, S., Meyer, D., and Gibson, G. R. (2007). A double-blind placebo-controlled study to establish the bifidogenic dose of inulin in healthy humans. Int. J. Clin. Pract. 61, 1199–1205. doi: 10.1111/j.1479-1613.2006.00626.x

Konstandinova, T., Parvanova, D., Atanassova, A., and Dziukanov, D. (2002). Fruiting tolerant tobacco, transformed to accumulate amylolytic protease. Plant Sci. 163, 157–159. doi: 10.1016/S0168-9452(01)00909-0

Lamommene, W., Le Roy, K., Van Laere, A., Rahim, A., and Van den Ende, W. (2008). Crystal structures of Ane- cedus duchesne cell wall invertase mutants in complex with sucrose. J. Mol. Biol. 377, 578–595. doi: 10.1016/j.jmb.2007.12.074

Lamommene, W., Le Roy, K., Tian, S., Ver- gauwen, R., Rahim, A., Van Laere, A., et al. (2012). Crystal structure of a 6-SST/6-SFT from Pachysandra ter- nomas, a plant fructan biosynthes- ing enzyme in complex with its acceptor substrate 6-hexose. Plant J. 70, 211–219. doi: 10.1111/j.1365-3040.2010.04401.x

Lassoued, B., Lotfi, J., Wierum, A., Van den Ende, W., et al. (2011). Towards a better understanding of the generation of fructan structure diversity in plant molecular and functional characterization of a novel fructan 6-fructofuranosyltransferase (6-SFT) cDNA from perennial ryegrass (Lolium perenne). Eur. J. Clin. Nutr. 65, 1871–1885. doi: 10.1038/ejcn.2011.388

Lawrence, B., Lottsch, E., Wannem, A., Van den Ende, W., et al. (2011). Towards a better understanding of the generation of fructan structure diversity in plant molecular and functional characterization of a novel fructan 6-fructofuranosyltransferase (6-SFT) cDNA from perennial ryegrass (Lolium perenne). Eur. J. Clin. Nutr. 65, 1871–1885. doi: 10.1038/ejcn.2011.388

Lee, J. B., Miyake, S., Uemura, R., Hara, K., Chitu, T., and Hayashi, T. (2012). Anti-influenza A virus effects of fructan from Welsh onion (Allium fistulosum L.). Jour. Amer. 156, 2164–2168. doi: 10.1016/j.jfoodbio.2012.04.010

Le Roy, K., Lamommene, W., Van Laere, A., and Van den Ende, W. (2008). Influencing the binding configuration of sucrose in the active site of chico fructan 1,2-fructosyltransferase 6: cocrystal structures of sucrose:fructan6:fructan6. New Phytol. 175, 572–586. doi: 10.1111/j.1469-8137.2008.02856.x
Maleux, A. D. (2002). Rubisco small subunit in plants.

Leslie, M. (2013). ‘Dead’ enzymes show cell type specificity and induction by stress.

Matrai, J., et al. (2013). Understanding the role of defective invertase in plants. 

Oliver, M. J., Tuba, Z., and Mishler, P., et al. (2007). Unraveling fructan biosynthesis in wheat.

Peshev, D., Vagena, R., Mogila, A., Hídy, E., and Van den Ende, W. (2013). Towards understanding vascular antimitotic mechanism.

Pesce, E., Eptet, A., Siepert, and Keller, F. (2010). Functional identification of Arabidopsis AT3IP2 (At3g57520) as an alkaloid α-galactosidase with a substrate specificity for raffinose and an apparent endo-α-galactosidase expression pattern.

Peshev, D., Vagena, R., Mogila, A., Hídy, E., and Van den Ende, W. (2013). Towards understanding vascular antimitotic mechanism.

Pilson-Smith, L. A. H., Blokamp, M. J. M., Paul, M. J., Jesek, M. J. W., Niebrücker, P. J., and Sméekens, S. C. M. (1995). Improved performance of transgenic fructose-fed cattle. 

Ruan, Y.-L., Patrick, J. W., Bouzayen, M., and Van den Ende, W. (2008). Creating S-type characteristics in the F-type enzyme fructan/fructan-1-fructosyltransferase of Trichoreutes auricots. 

Schneider, T., Kellen, F. (2009). Raffinose in chloroplasts is synthesized in the cytosol and transported across the chloroplast envelope.

Schroeven, L., Lammens, W., Kawakami, A., Yoshida, M., Van Laere, A., and Van den Ende, W. (2009). Creasing S-type characteristics in the F-type enzyme fructan/fructan-1-fructosyltransferase of Trichoreutes auricots. 

Stoyanova, S., Geuns, J., Hideg, E., and Van den Ende, W. (2011). The role of defective invertase in plants. 

Van den Ende, W., Stransky, H., and Schöffl, A. D. (2002). Rubisco small subunit in plants.

Verhaest, W., Stransky, H., and Schöffl, A. D. (2002). Rubisco small subunit in plants.

Wang, L., Zhang, H. L., Hansen, J., Sméekens, S. C. M., et al. (2011). Fructose sensitivity is suppressed in the elongating leaf bases in the Lolium perenne species.

Yamaguchi, K., and Ogawa, D., Yamaguchi, K., and Ogawa, D., Yamaguchi, K., and Ogawa, D. (2007). Unraveling fructan biosynthesis in wheat.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

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Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.

Zhang, J., Gao, F., and Zhang, J.-R. (2007). Improving freezing tolerance of transgenic tobacco expressing sucrose: a single amino acid.
Van den Ende, W., Clerens, S., Vergauwen, R., Van Riet, L., Van Laere, A., Yoshida, M., et al. (2005). Fruc-tan 1-sucralohydrolase (2,1) trim-mer during graminan biosynthesis in stoma of wheat (Triticum aestivum L.: Fru.) purification, characteri-sation, mass mapping and cloning of two fracture 1-embulbiose iso-meres. Plant Cell Environ. 28, 647–657. doi: 10.1111/j.1365-3040.2005.01328.x

Van den Ende, W., De Roeber, L., De Roover, J., Van Laere, A., Roy, K., Lammens, W., et al. (2002a). Unexpted presence of CBM for vacuolar invertase in wheat (Triticum aestivum L.: Fru.)-sucrose by sucrose, “in Plant Metabolism in Cereals: Wheat”, more than a Reserve Carbohydrate, Plant Physiol. 130, 1203–1213. doi: 10.1104/pp.116.2.709

Van den Ende, W., Clerens, S., Yashida, M., et al. (2001). Fructan 1-sucralohydrolase (2,1) trim-mer during graminan biosynthesis in stoma of wheat (Triticum aestivum L.: Fru.) characterization of its headgroups. J. Exp. Bot. 52, 1017–1027. doi: 10.1093/jxb/erf067

Van den Ende, W., Clerens, S., Vergauwen, R., Van Riet, L., Van Laere, A., Yoshida, M., et al. (2005). Fruc-tan 1-sucralohydrolase (2,1) trim-mer during graminan biosynthesis in stoma of wheat (Triticum aestivum L.: Fru.) characterization of its headgroups. J. Exp. Bot. 52, 1017–1027. doi: 10.1093/jxb/erf067

Van den Ende, W., Clerens, S., Vergauwen, R., Van Riet, L., Van Laere, A., Yoshida, M., et al. (2005). Fruc-tan 1-sucralohydrolase (2,1) trim-mers during graminan biosynthesis in stoma of wheat (Triticum aestivum L.: Fru.) purification, characteri-sation, mass mapping and cloning of two fracture 1-embulbiose iso-meres. Plant Cell Environ. 28, 647–657. doi: 10.1111/j.1365-3040.2005.01328.x

Van den Ende, W., De Roeber, L., De Roover, J., Van Laere, A., Roy, K., Lammens, W., et al. (2002a). Unexpted presence of CBM for vacuolar invertase in wheat (Triticum aestivum L.: Fru.)-sucrose by sucrose, “in Plant Metabolism in Cereals: Wheat”, more than a Reserve Carbohydrate, Plant Physiol. 130, 1203–1213. doi: 10.1104/pp.116.2.709

Van den Ende, W., Clerens, S., Yashida, M., et al. (2001). Fructan 1-sucralohydrolase (2,1) trim-mer during graminan biosynthesis in stoma of wheat (Triticum aestivum L.: Fru.) characterization of its headgroups. J. Exp. Bot. 52, 1017–1027. doi: 10.1093/jxb/erf067
Yuan, S., Le Roy, K., Venken, T., Lamens, W., Van den Ende, W., and De Maeyer, M. (2012). pKa modulation of the acid/base catalyst within GH52 and GH68: a role in substrate/inhibitor specificity? PLoS One 7:e37455. doi: 10.1371/journal.pone.0037455

Zhang, J., Dulk, B., Consensos, E., Weiers, I., Setzer, T., and Appels, R. (2008). Water deficits in wheat fructan exohydrolase (1-FEH) mRNA expression and relationship to soluble carbohydrate concentrations in two varieties. New Phytol. 181, 843–850. doi: 10.1111/j.1469-8137.2008.02713.x

Zimmermann, M. H., and Ziegler, H. (1975). “List of sugars and sugar alcohols in sieve-tube exudates,” in Transport in Plants: Phloem Transport. Encyclopedia of Plant Physiology, New Series, Vol. 1, eds M. H. Zimmermann, J. A. Milburn (New York: Springer), 480–503. doi: 10.1104/pp.108.134791

Zuther, E., Kwart, M., Willmitzer, L., and Heyer, A. G. (2004). Expression of a yeast-derived invertase in companion cells results in long-distance transport of a trisaccharide in an apoplastic loader and influences sucrose transport. Planta 218, 759–766. doi: 10.1007/s00425-003-1148-7

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