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OPINION PAPER

# Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging?

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## Abstract

**In nature, no single plant completes its life cycle without encountering environmental stress. When plant cells surpass stress threshold stimuli, chemically reactive oxygen species (ROS) are generated that can cause oxidative damage or act as signals. Plants have developed numerous ROS-scavenging systems to minimize the cytotoxic effects of ROS. The role of sucrosyl oligosaccharides (SOS), including fructans and the raffinose family oligosaccharides (RFOs), is well established during stress physiology. They are believed to act as important membrane protectors *in planta*. So far a putative role for sucrose and SOS during oxidative stress has largely been neglected, as has the contribution of the vacuolar compartment. Recent studies suggest a link between SOS and oxidative defence and/or scavenging. SOS might be involved in stabilizing membrane-associated peroxidases and NADPH oxidases, and SOS-derived radicals might fulfil an intermediate role in oxido-reduction reactions taking place in the vicinity of membranes. Here, these emerging features are discussed and perspectives for future research are provided.**

**Key words:** Fructan, oxidative stress, raffinose, ROS, sucrose, sucrosyl oligosaccharides.

## Introduction

Plant cells are challenged with hyperactive compounds derived from oxygen, the so-called reactive oxygen species (ROS) (Mittler *et al.*, 2004; Couée *et al.*, 2006). ROS include singlet oxygen ($^1O_2$), superoxide oxygen ($O_2^{\cdot-}$), hydroxyl radical (OH·), and hydrogen peroxide ($H_2O_2$), generated as by-products of photosynthesis and respiration (Mittler *et al.*, 2004). ROS production is directly connected to many metabolic processes in various subcellular compartments, especially chloroplasts, peroxisomes, and mitochondria (Fig. 1) (Bartoli *et al.*, 2004).

Chloroplasts and peroxisomes are the major ROS generators under excess light (Asada, 2006). It has been estimated that under normal physiological conditions, chloroplasts can generate ~150–250 μmol of $H_2O_2$ $mg^{-1}$ chlorophyll $h^{-1}$ (Wang and Song, 2008). Mitochondria can produce $O_2^{\cdot-}$ in the dark (Bartoli *et al.*, 2004; Møller *et al.*, 2007), rapidly inducing mitochondrial morphology transitions and leading to cell death (Scott and Logan, 2008). In addition, NADPH oxidases release ROS in the apoplast (Bolwell *et al.*, 2002).

The participation of the vacuole in oxidative stress has been totally neglected by most authors, but not by all (Mittler *et al.*, 2004). However, it should be realized that vacuoles occupy >95% of the cell volume in many plant cells. Moreover, the vacuole/tonoplast shows unusual structural adaptations under stress, triggering several stress-defensive mechanisms (Valluru *et al.*, 2008). Accordingly, vacuoles accumulate a mixture of strong antioxidant compounds (anthocyanins, phenolics, malate etc.; Kytridis and Manetas, 2006), probably fulfilling unanticipated roles in redox buffering.

ROS production can be accelerated by various environmental stresses, leading to lipid peroxidation and photo-oxidative damage (Murata *et al.*, 2007; Takahashi and Murata, 2008). These stresses have different effects on antioxidants (Kellos *et al.*, 2008). Stress stimuli can reduce $CO_2$ fixation, and impair net consumption of ATP and NADPH, generating singlet oxygen and causing photodamage to photosystem II (PSII; Hideg *et al.*, 2002). The

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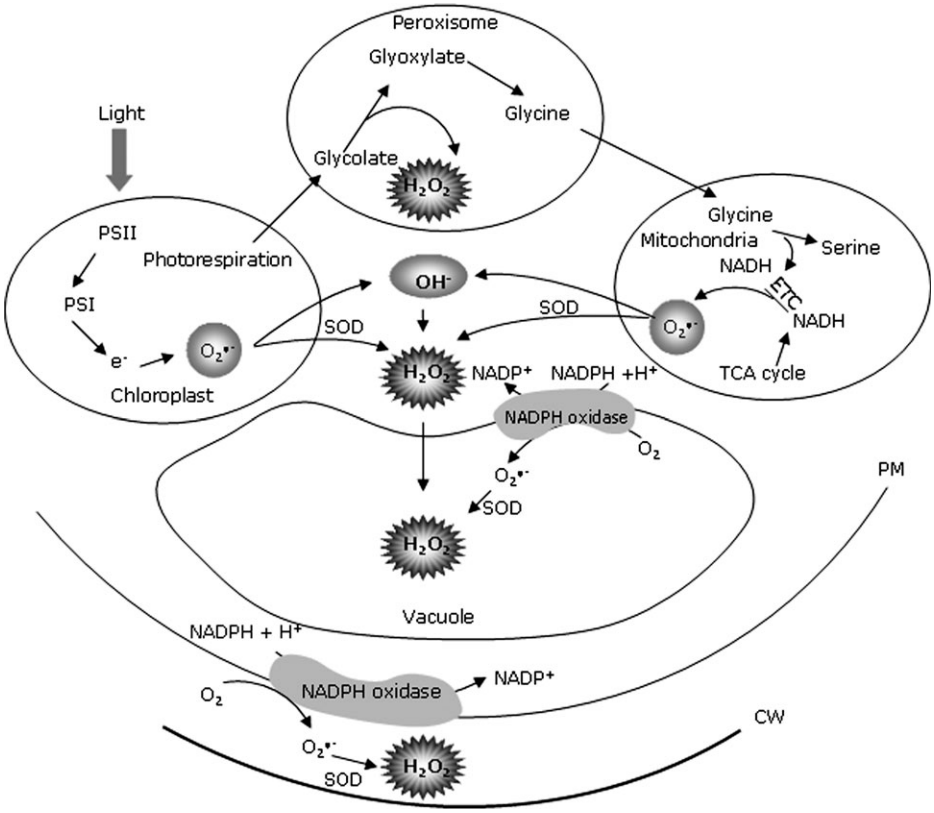


**Fig. 1.** Various intracellular sources of ROS ($H_2O_2$) in a plant cell. CW, cell wall; ETC, electron transport chain; $H_2O_2$ hydrogen peroxide; OH· hydroxyl radical; $O_2^{\bullet-}$, superoxide ion; PS, photosystems I and II; PM, plasma membrane; SOD, superoxide dismutase; TCA, tricarboxylic acid cycle.

increase in ROS concentration, in turn, activates antioxidants as well (Foyer and Noctor, 2005). Interestingly, stress stimuli seem to accelerate the photodamage to PSII by inhibiting its repair (Nishiyama *et al.*, 2006; Murata *et al.*, 2007; Takahashi and Murata, 2008). However, due to their sessile lifestyle, plants have developed a plethora of mechanisms to minimize oxidative damage under stress (see below).

In addition to well-known antioxidants, antioxidative defence systems and proteasome-dependent proteolytic systems (Møller *et al.*, 2007; Xiong *et al.*, 2007), small water-soluble sugars such as glucose and sucrose are now recognized as crucial compounds in coordinating plant developmental responses under oxidative stresses. In addition, other important water-soluble carbohydrates derived from sucrose [sucrosyl oligosaccharides (SOS)] include raffinose family oligosaccharides (RFOs) and fructans. As well as their role as sources of carbon and energy, which can back up growth and development during impaired metabolic activity, SOS have been assigned versatile regulatory functions at both the cellular and whole-organism level by controlling cellular metabolism, growth and development, and stress resistance of plants (Nishizawa *et al.*, 2008; Valluru and Van den Ende, 2008).

SOS and the enzymes associated with their metabolism might interact in indirect ways with ROS signalling pathways. Indeed, small soluble sugars and the enzymes associated with their metabolic pathways are widely believed to be connected to oxidative stress and ROS signalling pathways (Couée *et al.*, 2006; Sulmon *et al.*, 2006; Suzuki and Mittler, 2006; Takahashi and Murata, 2008). Furthermore, it cannot be excluded that fructans and RFOs themselves might act as signals in pathways associated with stress tolerance (Van den Ende *et al.*, 2004).

Here, the putative direct roles of SOS as primary ROS scavengers in the vicinity of cellular membranes, in close association with other key role players in antioxidative defence systems, are discussed. The modulating effects of fructans and RFOs are consistent with many observations scattered throughout the literature.

## SOS: a role in stress physiology

Raffinose, a α-galactosyl extension of sucrose, is nearly ubiquitous in plants (Keller and Pharr, 1996). The smallest RFOs, raffinose and stachyose, are synthesized in the cytoplasm. Both depend on galactinol [α-D-Gal-(1→1)-L-*myo*-inositol], the product of galactinol synthase (GolS). Raffinose synthase (RafS) catalyses the reversible transfer of a galactosyl unit from galactinol (donor substrate) to sucrose (acceptor substrate) (Lehle and Tanner, 1973). Subsequently, raffinose is used as an acceptor in the galactinol-dependent stachyose biosynthetic reaction catalysed by stachyose synthase (Peterbauer *et al.*, 1998). In contrast, the syntheses of

the higher DP (degree of polymerization) RFOs (>DP 4) are galactinol independent. The enzyme galactan:galactan galactosyltransferase (GGT) catalyses the direct transfer of a terminal galactosyl residue from one RFO molecule to another, resulting in the next higher and lower RFO oligomers, respectively (Haab and Keller, 2002; Tapernoux-Luthi *et al.*, 2004).

Fructans are sucrose-derived fructose polymers occurring in ~15% of flowering plants (Hendry, 1993) as well as in a wide range of bacteria and fungi (Martinez-Fleites *et al.*, 2005). Fructans are believed to be synthesized in the central vacuole (Frehner *et al.*, 1994), but an involvement of pre-vacuolar vesicles cannot be excluded (Kaeser 1983). Fructan biosynthesis is initiated by sucrose:sucrose 1-fructosyltransferase (1-SST), donating a fructosyl moiety from one sucrose to another (Edelman and Jefford, 1968; Van den Ende and Van Laere, 1996). This process yields the trisaccharide 1-kestose, the simplest inulin with β(2,1)-linkages, which can be elongated further by adding β(2,1)-and/or β(2,6)-linked fructosyl moieties by other fructosyl transferase (FT) enzymes such as 1-FFT, 6G-FFT, and 6-SFT. Depolymerization of fructans is executed by fructan exohydrolases (FEHs). Different types of FEHs (1-FEH, 6-FEH, 6-KEH, and 6&1-FEH) have recently been described in fructan- and non-fructan-containing plants (De Coninck *et al.*, 2007; Van Riet *et al.*, 2008).

Fructans fulfil protective physiological roles in plants (Hendry, 1993; Morvan-Bertrand *et al.*, 2001; Le Roy *et al.*, 2007). During stresses, fructans can strongly interact with cell membranes through direct hydrogen bonding (Hincha *et al.*, 2000, 2003). The surface-active effects of both inulin- and levan-type fructans contrast strongly with the maximal effects observed for trehalose, sucrose, and glucose. Inulin-type fructans show a deep interaction with membranes compared with levan-type fructans due to their variable molecular weight (Hinrichs *et al.*, 2001) and flexible random coil structures (Vereyken *et al.*, 2003). Fructans prevent lipid condensation and cessation of the phase transition by reducing the molecular motions of the lipid head groups (Vereyken *et al.*, 2003). RFOs fulfil similar physiological roles in plants, and were shown to be involved in desiccation tolerance in seeds (Keller and Pharr, 1996). Both RFOs and fructans are believed to protect biological membranes under stress (Hincha *et al.*, 2002, 2003).

## Links between oxidative stress and carbon metabolism

### *ROS and sugar signalling: a delicate balance*

Soluble sugars such as glucose and sucrose have long been considered to play versatile roles in plants (Rolland *et al.*, 2006). Recently, the remodelling of carbon metabolism in *Arabidopsis* is interpreted as an emergency strategy under oxidative stress (Scarpeci and Valle, 2008). Higher photosynthetic activity induces both the generation of ROS and massive accumulation of soluble sugars. Therefore, sugars themselves might be effective candidates for the oxidative burst in tissues exposed to a wide range of environmental stresses.

Endogenous sugar availability can feed the oxidative-pentose phosphate pathway (OPP; Debnam *et al.*, 2004; Couée *et al.*, 2006), which can trigger ROS scavenging. Glucose 6-phosphate dehydrogenase (G6PDH), catalysing the first reaction in the OPP pathway, has been postulated to affect the redox poise of the chloroplast as well as the capacity to detoxify ROS (Debnam *et al.*, 2004). Sugars can replenish NADPH, needed for monodehydroascorbate reductase (MDAR) and glutathione reductase (GR) (Nishikawa *et al.*, 2005). The effects of soluble sugars on gene expression are mediated through sugar-specific signalling pathways (Couée *et al.*, 2006). Interestingly, the responses to sugars and oxidative stress are not only co-linked, but also affect scores of stress-responsive genes (Price *et al.*, 2004). Moreover, sugar availability can enhance ascorbate (ASC) biosynthesis (Nishikawa *et al.*, 2005), perhaps due to the enhanced rate of respiration (Millar *et al.* 2003).

Conclusively, so far the protective effects of soluble sugars related to oxidative stress have been considered as indirect effects of sugar signalling, triggering the production of specific ROS scavengers.

### *Sucrose: an underestimated antioxidant capacity against ROS?*

*In vitro* studies demonstrated that the $ID_{50}$ values (the concentration of a compound required to inhibit OH·-catalysed hydroxylation of salicylate by 50% of the maximum yield observed in the absence of the compound) for galactinol (3.1 mM) and raffinose (2.9 mM) are similar to that of glutathione (GSH) (3.0 mM) and smaller than that of ASC (16.4 mM: Nishizawa *et al.*, 2008), two classical antioxidants. Strikingly, when compared with other sugars, the strongest antioxidant capability was detected for sucrose ($ID_{50}$: 2.7 mM), in line with earlier observations (Smirnoff and Cumbes, 1989). OH· are highly reactive radicals, which retrieve H· from virtually any organic compound to form water. In sugars, OH· preferentially attack HO–C–H linkages (Morelli *et al.*, 2003). Accordingly, when sugars are compared at the same molar concentration, their free radical-scavenging capacity is strongly correlated with their total number of hydroxyl groups, explaining why sucrose (eight OH groups) is better compared with glucose and fructose (five OH groups). In a similar vein, lower DP fructans as soluble polyhydroxy compounds might be even more efficient in radical quenching (see Fig. 2). The identity of the liberated oxidized sucrose free radicals (OSFRs) might be diverse, and their exact nature and stability deserve further investigation. However, OSFRs are slower reacting radicals compared with OH· radicals and seem to undergo several possible reactions to form more stable non-radical compounds or, alternatively, to regain their reduced forms (Green, 1980; Gray and Mower, 1991).

These *in vitro* studies convincingly demonstrate the ROS-scavenging capacity of sucrose, strongly suggesting that similar reactions can also occur *in planta*. At low

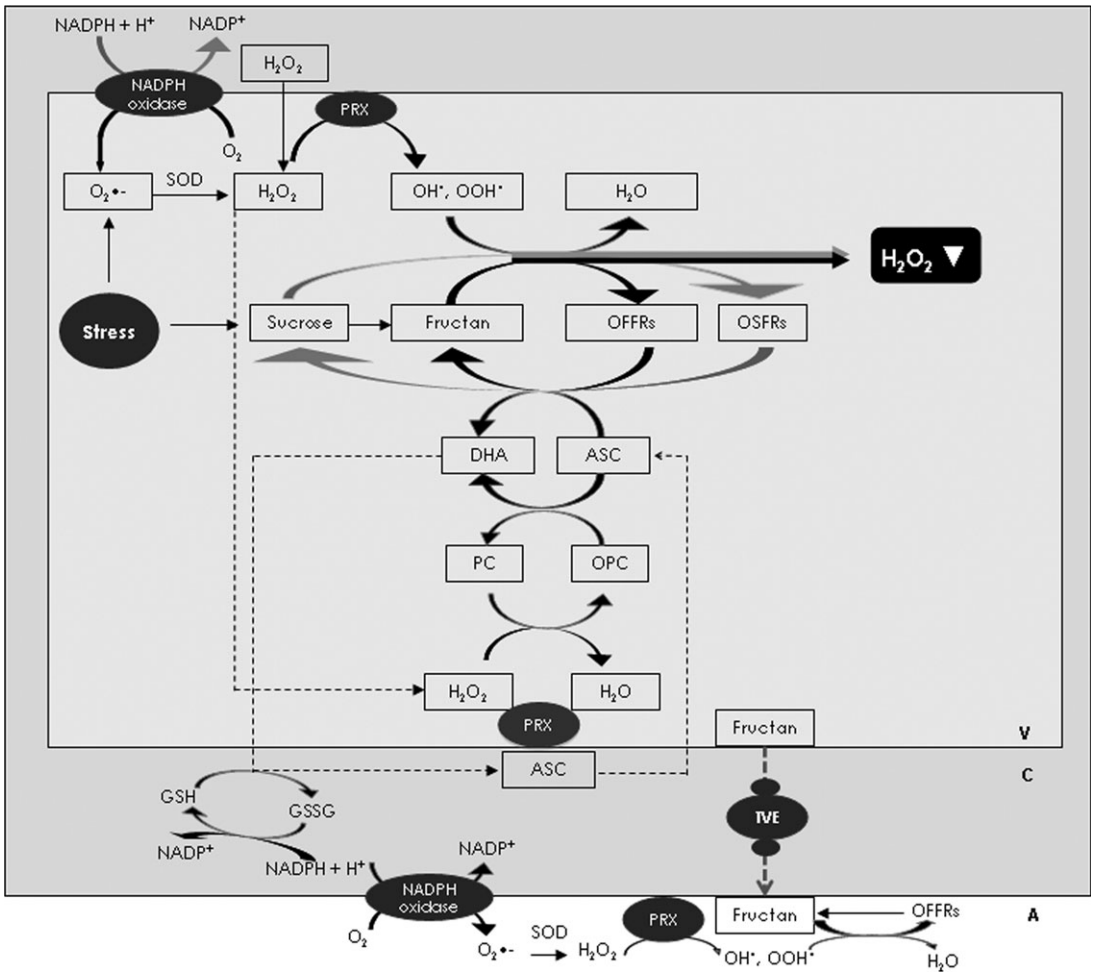


**Fig. 2.** Possible scavenging mechanisms of fructans and sucrose in oxidative stress defence. ASC, ascorbate; DHA, dehydroascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; $H_2O_2$, hydrogen peroxide; $O_2^{\cdot -}$, superoxide ion; OH· hydroxyl radical; OFFRs, oxidized fructan free radicals; OSFRs, oxidized sucrose free radicals; OPC, oxidized phenolic compounds; SOD, superoxide dismutase; PC, phenolic compounds; PRX, peroxidase; V, vacuole; C, cytoplasm; A, apoplast.

concentrations, sucrose might serve as a substrate or signal for stress-induced alterations, while at high concentrations it can function directly as a protective agent (Uemura and Steponkus, 2003). The mechanism of OH· scavenging might be linked to the presence of stable OSFRs. However, these sugar radicals may easily regenerate *in vivo* (see later), providing higher stability of the sucrose and a more efficient quenching of the OH·. These processes might be of particular importance in vacuoles of sugar-accumulating tissues such as sugar beet and sugar cane, in phloem-associated tissues, or in any cells with enhanced sucrose concentrations.

So far, sucrose has not been recognized as an antioxidant compound. One of the reasons for this is the fact that so far research efforts have been almost entirely focused on *Arabidopsis*. Quite exceptionally, when compared with most other plants, *Arabidopsis* contains very low sucrose concentrations that cannot be substantially elevated under mild stress conditions (own unpublished observations), suggesting that the sucrose concentration is rather strictly controlled in this species. Instead, *Arabidopsis* rapidly diverts excess carbon to RFOs (Klotke *et al.*, 2004) and/or to starch (Mita *et al.*, 1995).

### *ROS and RFOs: a link in* Arabidopsis

Recently, RFO sugars as well as galactinol have been proposed to fulfil important roles in oxidative stress defence in plants (Morsy *et al.*, 2007; Nishizawa *et al.*, 2008) and seeds (Buitink *et al.*, 2000; Bailly *et al.*, 2001; Lehner *et al.*, 2006). In *Arabidopsis*, seven genes belonging to the *GolS* family were identified, among these, GolS1 and 2 mRNAs were detected in mature seeds that were induced by stresses in leaf tissues, while GolS3 mRNA seems to be induced by cold stress (Panikulangara *et al.*, 2004). Overexpression of *GolS1*, *GolS2*, *GolS4*, and *RafS2* in transgenic *Arabidopsis* increased the galactinol and raffinose concentrations and resulted in effective ROS-scavenging capacity and oxidative stress tolerance (Nishizawa *et al.*, 2008). Concomitantly, the levels of the antioxidants ASC and GSH also increased. Moreover, lipid peroxidation was significantly lower than in wild-type plants (Nishizawa *et al.*, 2008). Further, these transgenic plants exhibited higher PSII activities, compared with wild types, and responded positively to high light and chilling conditions. These results strongly suggest that endogenous galactinol and raffinose can act as antioxidants/

osmoprotectants *in planta*, leading to increased tolerance to oxidative stress (methylviologen treatment).

Chloroplasts generate massive ROS under stress. $O_2^{\cdot -}$, as an initial ROS, is readily converted into OH· and $H_2O_2$. This stimulates a battery of antioxidant systems capable of removing ROS from the chloroplasts, such as flavonoids (Agati *et al.*, 2007), and ASC and GSH (Asada, 2006). The accumulation of raffinose in chloroplasts (Santarius and Milde, 1977; Lineberger and Steponkus, 1980; Heber and Heldt, 1981) indicates that raffinose transporters (cf. ASC and GSH transporters; Pignocchi and Foyer, 2003) might exist in chloroplast membranes (Heber and Heldt, 1981) but so far they have not been characterized. Similarly, the question of whether sucrose is present inside plastids has long been debated. Gerrits *et al.* (2001) have introduced sucrose-metabolizing enzymes into plastids. These experiments suggested substantial sucrose entry into plastids. Previously, raffinose was shown to protect photophosphorylation and electron transport of chloroplast membranes against freezing, desiccation, and high temperature stress (Santarius, 1973), strongly suggesting that chloroplastic RFOs might be operating as ideal ROS scavengers. The oxidized RFO radicals might be regenerated by ASC or other reducing antioxidants such as flavonoids (Agati *et al.*, 2007).

### *ROS and fructans: a new link?*

Mounting evidence has been generated over the last decade that fructans might protect plants against freezing/drought stresses (Hincha *et al.*, 2000, 2003). The putative roles of fructans localized in the vacuole (Kawakami *et al.*, 2008) and in the apoplast (Van den Ende *et al.*, 2005; Valluru *et al.*, 2008) were established, and a role in oxidative stress defence has been proposed (Parvanova *et al.*, 2004). These studies suggest that fructans act directly as ROS scavengers (Fig. 2) or indirectly by stimulating other specific antioxidative defence mechanisms. Interestingly, changes in fructan concentrations showed a close relationship with changes in antioxidant (ASC and GSH) concentrations in immature wheat kernels (De Gara *et al.*, 2003; Paradiso *et al.*, 2006), strongly suggesting a link with well-known antioxidant systems, and may occupy an integral part of a complex ROS-scavenging concept.

So far, fructans are not recognized as 'typical' antioxidants in plants. However, hot water extracts of the fructan plants *Chlorophytum borivillianum* (Govindarajan *et al.*, 2005) and *Arctium lappa* (edible burdock: Duh, 1998) showed strong antioxidant properties, acting as effective radical scavengers in *in vitro* tests. Moreover, these extracts showed prominent bioactive properties in animal studies (Kardosova *et al.*, 2003). These data strongly suggest that vacuolar fructans, like vacuolar anthocyanins, could fulfil a role in redox regulation processes.

Since the vacuole harbours both peroxidases (Prx; Mittler, 2002; Sottomayor *et al.*, 2004) and fructans (Frehner *et al.*, 1984), a Prx-dependent oxidation of fructans seems possible in the vacuole of fructan-containing plants. Unlike ASC and phenolic compounds (PCs), fructans and other carbohydrates lack a double bond in their ring structure, which probably prevents them from acting as suitable substrates for Prx or oxidase enzymes. However, carbohydrate oxidase enzymes, oxidizing reducing sugars, have been characterized from fructan-containing plants (Custers *et al.*, 2004), but vacuolar forms that prefer non-reducing carbohydrates such as fructans have not yet been reported. In the absence of such evidence for specific fructan oxidase or Prx enzymes, it seems reasonable to speculate that fructan oxidation could be initiated by $O_2^{\cdot -}$, OH·, and OOH·, retrieving H· to form water and generating oxidized fructan free radicals (OFFRs).

Two mechanisms have been postulated to explain the origin of the initiator radicals in the vacuole, and these two systems are not mutually exclusive (Sottomayor *et al.*, 2004).

The first possibility is the diffusion of excess cytoplasmic $H_2O_2$ through the tonoplast. Tonoplastic aquaporins may facilitate $H_2O_2$ uptake (Reisen *et al.*, 2003; Bienert *et al.*, 2007). Independent studies on isolated tonoplast fractions have repeatedly demonstrated the presence of membrane-associated Prx or class III peroxidases [barley peroxidase gene (Prx7), Kristensen *et al.*, 2001; *Catharanthus roseus* peroxidase (CRPrx), Sottomayor and Ros Barcelo, 2003; *Arabidopsis thaliana* peroxidase (AtPrx34), Zimmermann *et al.*, 2004; *Catharanthus roseus* peroxidase 1 (CrPrx1), Costa *et al.*, 2008]. Importantly, these peroxidases are localized on the inner face of the tonoplast (Sottomayor *et al.*, 2004), which can readily attack incoming $H_2O_2$, generating a blend of ROS (OH· and OOH·) by the hydroxylic cycle of these peroxidases (Passardi *et al.*, 2004; Dunand *et al.*, 2007). At the same time, these radicals might oxidize many vacuolar compounds likely to complement the classical ASC–ascorbate peroxidase (APX) system (Yamasaki and Grace, 1998; Grace and Logan, 2000).

A second possible mechanism of ROS generation involves the action of a tonoplastic NADPH oxidase. These enzymes are considered as major ROS producers in the plasma membrane (PM), but proteomic studies have documented the presence of these enzymes in the tonoplast as well (Carter *et al.*, 2004; Whiteman *et al.*, 2008). Consistently, $O_2^{\cdot -}$, the first product generated by this enzyme, has been detected in the tonoplast (Romero-Puertas *et al.*, 2004). Taken together, it can be hypothesized that a tonoplastic NADPH oxidase might use the cytoplasmic NADPH to transfer electrons across the membrane to form $O_2^{\cdot -}$, as described in lysosomes (Chen, 2002) and phagocytic vacuoles in animal cells (Behe and Segal, 2007). This $O_2^{\cdot -}$ could be transformed to the less toxic $H_2O_2$ spontaneously or via tonoplast-associated superoxide dismutase (SOD) (Shi *et al.*, 2007).

After generation by membrane-bound oxidases and peroxidases, ROS present a great danger for these membranes (lipid peroxidation). Fructans can protrude deep into membranes (deeper than sucrose) as described (Valluru and Van den Ende, 2008), contributing to membrane stabilization. It is hypothesized that these membrane-associated fructans might also be ideally positioned to react with these radicals, to form OFFRs, in this way preventing lipid peroxidation (Fig. 2). However, these OFFRs might be

rapidly reduced again to fructans by the 'classical' antioxidant ASC or by other vacuolar antioxidants (PCs and anthocyanins). Such an 'NADPH oxidase/Prx/fructan/PC' system within the tonoplast (NADPH oxidase), associated with the inner side of the tonoplast (Prx/fructan/PC), and present in the vacuolar lumen (fructan/PC) could be elegantly linked with the cytoplasmic redox systems (Fig. 2). It may operate as a unique scavenging and salvaging system, preventing lipid degradation, in this way ensuring membrane stabilization and contributing to cell survival by removing excess $H_2O_2$ that is formed in or diffused into vacuoles. Indeed, it has been shown that plant cell viability depends on the functional status of the vacuole and intact vesicular trafficking (Surpin and Raikhel, 2004). A similar scavenging system has also been proposed before for phenoxy radicals (Mehlhorn *et al.*, 1996; Takahama, 2004). Recently, a role for trehalose in protection against ROS was also demonstrated (Nery *et al.*, 2008).

Regeneration of OFFRs into fructans might be an important aspect for efficient quenching of ROS. Vacuolar compounds such as flavonoids (e.g. anthocyanins) might be involved in reduction of OFFRs into fructans. Indeed, flavonoids may also act as antioxidants (Kytridis and Manetas, 2006; Pourcel *et al.*, 2007) and a strong correlation was found between flavonoid content and freezing tolerance (Korn *et al.*, 2008). Previous knock-out experiments revealed that raffinose alone could not account for the observed freezing tolerance (Zuther *et al.*, 2004). It is proposed here that perhaps the *combination* of sugars and (different) flavonoids might be essential to establish freezing tolerance in this species. Indeed, conjugated flavonoid compounds have been shown to have a stronger scavenging effect on ROS than their respective monomers, and thus seem to moderate the pro-oxidant properties of antioxidants (Kang, 2007). As a new concept, it can be hypothesized that both sugars and phenolic compounds form part of an integrated redox system, quenching ROS and contributing to freezing tolerance (see further Fig. 2).

It should be noted that under stress, a tonoplast vesicle-derived exocytosis (TVE) (Valluru *et al.*, 2008) might be operating as an efficient system to carry fructans (and sucrose) from the vacuole to the PM in plants (Fig. 2). A similar vesicular transport from lysosomes to the cell surface was described in animal cells (Wubbolts *et al.*, 1996). Therefore, and perhaps even more importantly, a very similar system involving PM-localized NADPH oxidase and soluble sugars such as fructans (and perhaps Prx: Mika and Lúthje, 2003) might fulfil significant roles in preserving PM stability. Such a system might greatly contribute to stress tolerance and signalling pathways controlling apoplastic $H_2O_2$ concentrations, regulating defence responses (Orozco-Cardenas *et al.*, 2001) as well as growth and development by cell wall modifications (Passardi *et al.*, 2004).

The model depicted in Fig. 2 depends on high sucrose concentrations (as a substrate for fructan biosynthesis by FTs), oxygen availability, and on the presence of PCs. Consistent with the present model, much higher fructan levels are generated under hypoxia (Albrecht *et al.*, 2004), to keep OFFR levels high despite the reduced OH· generation. However, the model cannot work under complete anoxia. Indeed, it was found that fructans are totally degraded under these circumstances (Albrecht *et al.*, 2004). Consistent with the model, $H_2O_2$ seems to accumulate in vascular tissues such as leaf veins (Fryer *et al.*, 2002; Slesak *et al.*, 2008). Strikingly, phloem-associated tissues typically form a major site of storage for several sugar compounds, including sucrose and fructans (Wang and Nobel, 1998; Van den Ende *et al.*, 2000).

The relevance of the concept might be validated from the studies carried out on transgenic non-fructan tobacco plants carrying FTs (SacB gene, Konstantinova *et al.*, 2002; Parvanova *et al.*, 2004; 1-SST, Li *et al.*, 2007) which showed more resistance to frost. Closer observations elucidated that transformants are able to maintain oxidative compounds such as malondialdehyde–an end-product of lipid peroxidation and $H_2O_2$–within the controlled range to cope with oxidative damage (Parvanova *et al.*, 2004; Li *et al.*, 2007).

So far, the reasons behind the partial degradation of fructans in cold-induced (0–5 °C) autumn chicory roots, a very well studied physiological response (Van Laere and Van den Ende, 2002), remained obscure. Indeed, these growth-arrested plants do not need carbon skeletons or osmotic adjustments. According to the hypothesis presented here, it is now proposed that the partial degradation of longer DP fructans increases the total number of molecules (fructose, sucrose, and lower DP fructans) to increase scavenging capacities and deal with the increased oxidative stress under chilling. Indeed, longer DP fructans might be too extended, part of these molecules being too far away from the tonoplast, the actual place of ROS generation. Similarly, the introduction of yeast invertase in potato increased the sugar concentration, contributing to chilling tolerance (Deryabin *et al.*, 2007). Supporting the same idea, the breakthrough manuscript of Kawakami *et al.* (2008), introducing wheat 1-SST in the non-fructan plant rice, convincingly demonstrated that transgenic rice plants became more tolerant to chilling. The stabilizing effect of fructans on membranes might be of crucial importance during freezing (subzero temperatures) but probably not during chilling. Therefore, the proposed ROS-scavenging concept could probably explain the chilling tolerance observed in transgenic rice. Strikingly, both 1-SST introduction (Kawakami *et al.*, 2008) and heat shock-mediated APX expression (Sato *et al.*, 2001) can protect rice plants against chilling injury.

## Conclusions and perspectives

Exposure to environmental stress often results in increased production of ROS in plants. The plant's capacity to delineate these toxic compounds depends on the metabolic responsiveness of defensive mechanisms. Both enzymatic and non-enzymatic defence pathways can detoxify ROS. Sucrose and SOS (including fructans and RFOs) fulfil various functional roles in plant metabolism. SOS might either directly detoxify ROS in chloroplasts and vacuoles or indirectly stimulate the classic antioxidative defence

systems. As a new concept, it can be hypothesized that the synergistic interaction of SOS and phenolic compounds forms part of an integrated redox system, quenching ROS and contributing to stress tolerance, especially in tissues with high soluble sugar concentrations. However, the exact chemical identity and stability of the SOS radicals remain obscure and need further exploration. Furthermore, it can be expected that SOS-related scavenging mechanisms would affect ROS signalling pathways. How such signalling cascades control the survival, or death, of plants would be a fascinating perspective.

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