

OPINION PAPER

Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging?Wim Van den Ende^{1,*} and Ravi Valluru²¹ Laboratory for Molecular Plant Physiology, KU Leuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium² Institute for Crop Production and Grassland Research, University of Hohenheim, D-70599 Stuttgart, Germany

Received 25 September 2008; Revised 20 October 2008; Accepted 23 October 2008

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 (¹O₂), superoxide oxygen (O₂^{•-}), hydroxyl radical (OH[•]), and hydrogen peroxide

(H₂O₂), 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 H₂O₂ mg⁻¹ chlorophyll h⁻¹ (Wang and Song, 2008). Mitochondria can produce O₂^{•-} 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 CO₂ fixation, and impair net consumption of ATP and NADPH, generating singlet oxygen and causing photodamage to photosystem II (PSII; Hideg *et al.*, 2002). The increase in ROS concentration, in turn, activates

* To whom correspondence should be addressed. E-mail: wim.vandenende@bio.kuleuven.be

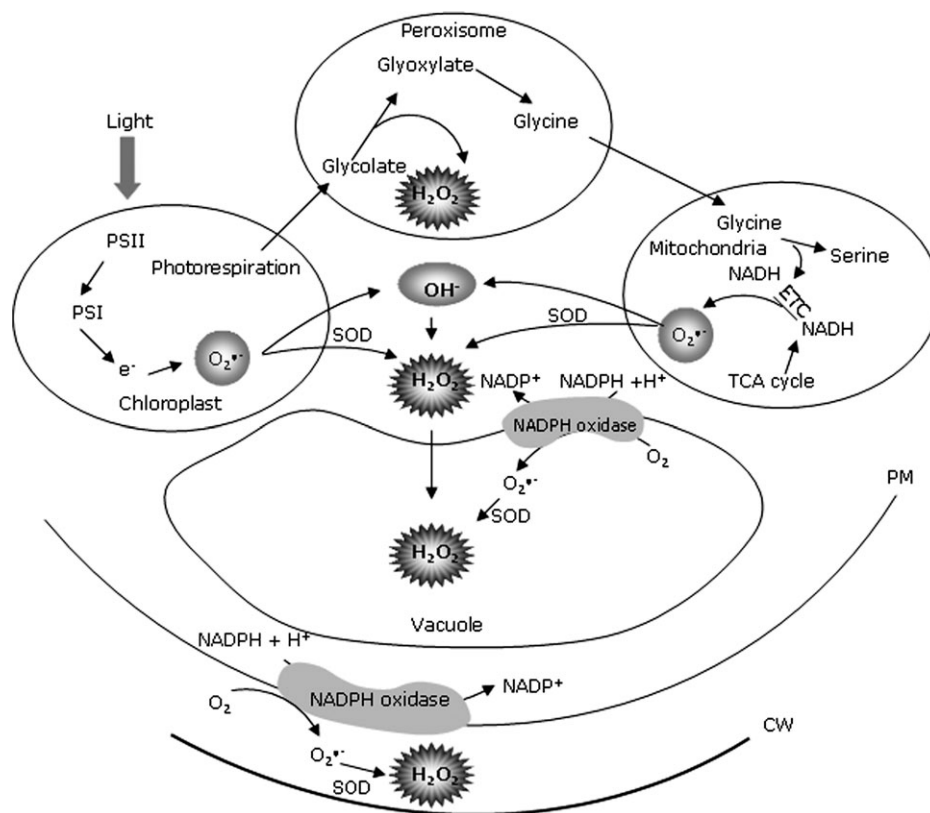


Fig. 1. Various intracellular sources of ROS (H_2O_2) in a plant cell. CW, cell wall; ETC, electron transport chain; H_2O_2 hydrogen peroxide; OH^\cdot hydroxyl radical; $\text{O}_2^{\cdot-}$, superoxide ion; PS, photosystems I and II; PM, plasma membrane; SOD, superoxide dismutase; TCA, tricarboxylic acid cycle.

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 \rightarrow 1)-L-*myo*-inositol], the product of galactinol synthase (GalS). 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 (Kaesler 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 $\beta(2,1)$ -linkages, which can be elongated further by adding $\beta(2,1)$ - and/or $\beta(2,6)$ -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 ID₅₀ 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 (ID₅₀: 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

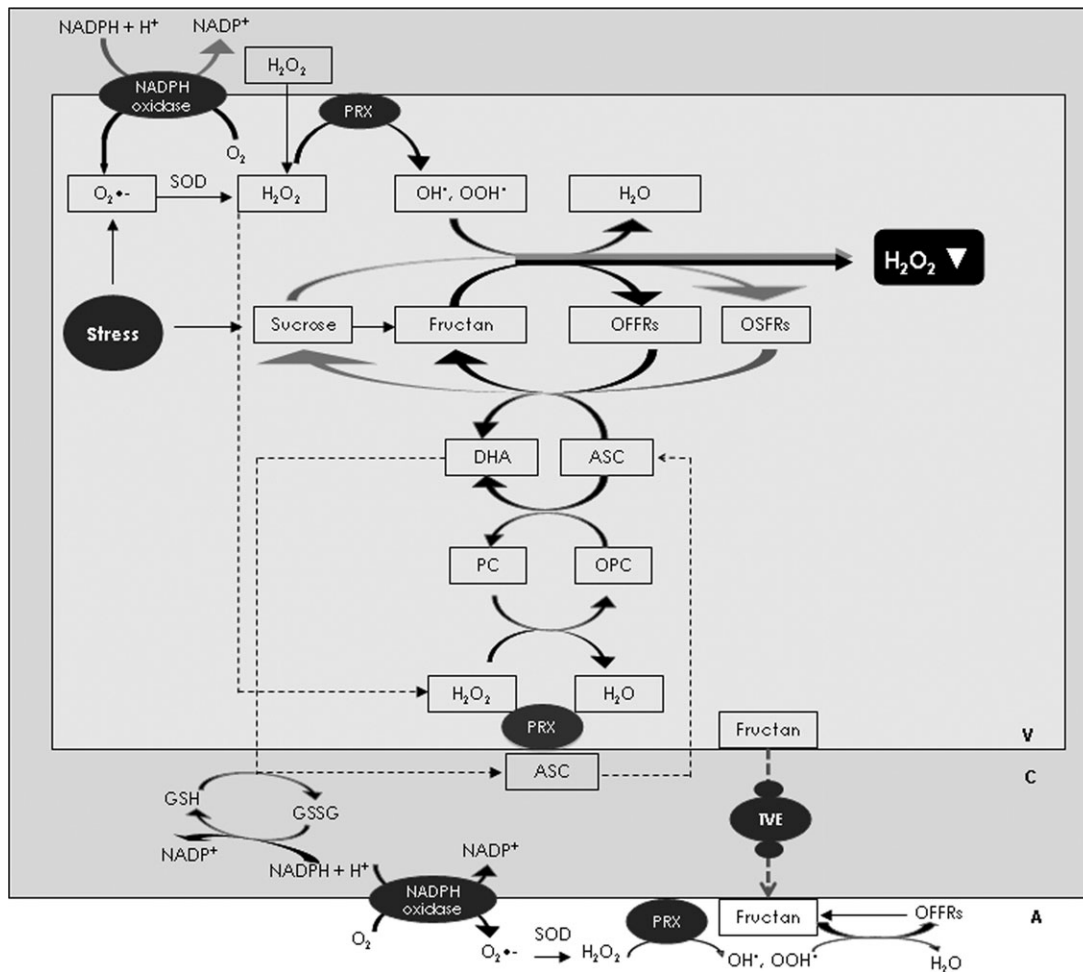


Fig. 2. Possible scavenging mechanisms of fructans and sucrose in oxidative stress defence. ASC, ascorbate; DHA, dehydroascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; H₂O₂, hydrogen peroxide; O₂^{•-}, superoxide ion; OH[•], hydroxyl radical; OFFRs, oxidized fructan free radicals; OSFRs, oxidized sucrose free radicals; OPC, oxidized phenolic compounds; SOD, superoxide dismutase; PC, phenolic compounds; PRX, peroxidase; V, vacuole; C, cytoplasm; A, apoplast.

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 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). Over-expression 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 $OH\cdot$ 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 borivillanum* (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 $O_2^{\cdot-}$, $OH\cdot$, and $OOH\cdot$, retrieving $H\cdot$ 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 ($OH\cdot$ and $OOH\cdot$) 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 tonoplast 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 tonoplast 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 experi-

ments 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.

Acknowledgements

The authors thank Professor A. Van Laere for critically reviewing this manuscript. RV is funded by the University of Hohenheim and Baden-Württemberg state, Germany. WVdE is supported by grants from FSR Flanders.

References

- Agati G, Matteini P, Goti A, Tattini M. 2007. Chloroplast-located flavonoids can scavenge singlet oxygen. *New Phytologist* **174**, 77–89.
- Albrecht G, Mustroph A, Fox TC. 2004. Sugar and fructan accumulation during metabolic adjustment between respiration and fermentation under low oxygen conditions in wheat roots. *Physiologia Plantarum* **120**, 93–105.
- Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology* **144**, 391–396.
- Bailly C, Audigier C, Ladonne F, Wagner MH, Coste F, Corbineau F, Côme D. 2001. Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. *Journal of Experimental Botany* **52**, 701–708.
- Bartoli CG, Gómez F, Martínez DE, Guiamet JJ. 2004. Mitochondria are the main target for oxidative damage in leaves of wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **55**, 1663–1669.
- Behe P, Segal AW. 2007. The function of the NADPH oxidase of phagocytes, and its relationship to other NOXs. *Biochemical Society Transactions* **35**, 1100–1103.
- Bienert GP, Møller ALB, Kristiansen KA, Schulz A, Møller IM, Schjoerring JK, Jahn TP. 2007. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *Journal of Biological Chemistry* **282**, 1183–1192.
- Bolwell GP, Bindschedler LV, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, Minibayeva F. 2002. The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. *Journal of Experimental Botany* **53**, 1367–1376.
- Buitink J, Hemminga MA, Hoekstra FA. 2000. Is there a role for oligosaccharides in seed longevity? An assessment of intracellular glass stability. *Plant Physiology* **122**, 1217–1224.
- Carter C, Songqin P, Zouhar J, Avila EL, Girke T, Raikhel NV. 2004. The vegetative vacuole proteome of *Arabidopsis thaliana* reveals predicted and unexpected proteins. *The Plant Cell* **16**, 3285–3303.
- Chen CS. 2002. Phorbol ester induces elevated oxidative activity and alkalization in a subset of lysosomes. *BMC Cell Biology* **3**, 21.
- Costa MMR, Hilliou F, Duarte P, Pereira LG, Almeida I, Leech M, Memelink J, Barceló AR, Sottomayor M. 2008. Molecular cloning and characterization of a vacuolar class III peroxidase involved in the metabolism of anticancer alkaloids in *Catharanthus roseus*. *Plant Physiology* **146**, 403–417.
- Couée I, Sulmon C, Gouesbet G, Amrani AE. 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *Journal of Experimental Botany* **57**, 449–459.
- Custers JH, Harrison SJ, Sela-Buurlage MB, *et al.* 2004. Isolation and characterisation of a class of carbohydrate oxidases from higher plants, with a role in active defence. *The Plant Journal* **39**, 147–160.
- De Coninck B, Van den Ende W, Le Roy K. 2007. Fructan exohydrolases (FEHs) in plants: properties, occurrence and 3-D structure. In: Shiomi N, Noureddine B, Shuichi O, eds. *Recent advances in fructooligosaccharides research*. Philadelphia, PA: Old City Publishers, 157–180.
- De Gara L, de Pinto MC, Moliterni VMC, D'Egidio MG. 2003. Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *Journal of Experimental Botany* **54**, 249–258.
- Debnam PM, Fernie AR, Lisse A, Golding A, Bowsher CG, Grimshaw C, Knight JS, Emes MJ. 2004. Altered activity of the P2 isoform of plastidic glucose-6-phosphate dehydrogenase in

- tobacco (*Nicotiana tabacum* cv. Samsun) causes changes in carbohydrate metabolism and response to oxidative stress in leaves. *The Plant Journal* **38**, 49–59.
- Deryabin AN, Sinkevich MS, Dubinina IM, Burakhanova EA, Trunova TI.** 2007. Effect of sugars on the development of oxidative stress induced by hypothermia in potato plants expressing yeast invertase gene. *Russian Journal of Plant Physiology* **54**, 32–38.
- Duh PD.** 1998. Antioxidant activity of burdock (*Arctium lappa* Linn): its scavenging effect on free radical and active oxygen. *Journal of the American Oil Chemistry Society* **75**, 455–461.
- Dunand C, Crévecoeur M, Penel C.** 2007. Distribution of superoxide and hydrogen peroxide in *Arabidopsis* root and their influence on root development: possible interaction with peroxidases. *New Phytologist* **174**, 332–341.
- Edelman J, Jefford TG.** 1968. The mechanism of fructosan metabolism in plants exemplified in *Helianthus tuberosus*. *New Phytologist* **67**, 517–531.
- Foyer CH, Noctor G.** 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell* **17**, 1866–1875.
- Frehner M, Keller F, Wiemken A.** 1984. Localization of fructan metabolism in the vacuoles isolated from protoplasts of Jerusalem artichoke. *Journal of Plant Physiology* **116**, 197–208.
- Fryer MJ, Oxborough K, Mullineaux PM, Baker NR.** 2002. Imaging of photo-oxidative stress responses in leaves. *Journal of Experimental Botany* **53**, 1249–1254.
- Gerrits N, Turk SCHJ, van Dun KPM, Hulleman SHD, Visser RGF, Weisbeek PJ, Smeekens SCM.** 2001. Sucrose metabolism in plastids. *Plant Physiology* **125**, 926–934.
- Govindarajan R, Sreevidya N, Vijayakumar M, Thakur M, Mehrotra S, Pushpangadan P.** 2005. *In vitro* antioxidants activity of ethanolic extract of *Chlorophytum borivillanum*. *National Proceedings of Science* **11**, 165–169.
- Grace SC, Logan BA.** 2000. Energy dissipation and radical scavenging by the plant phenolpanoid pathway. *Philosophical Transactions of the Royal Society B: Biological Sciences* **355**, 1499–1510.
- Gray J, Mower HF.** 1991. The role of simple carbohydrates in the suppression of hydroxyl free radicals in γ -irradiated papaya juice. *Food Chemistry* **41**, 293–301.
- Green JW.** 1980. Oxidative reactions and degradations. In: Pigman W, Horton D, Wander JD, eds. *The carbohydrates. Chemistry and biochemistry*, 2nd edn. New York: Academic Press, 1126–1135.
- Haab CI, Keller F.** 2002. Purification and characterization of the raffinose oligosaccharide chain elongation enzyme, galactan:galactan galactosyltransferase (GGT), from *Ajuga reptans* leaves. *Physiologia Plantarum* **114**, 361–371.
- Heber U, Heldt HW.** 1981. The chloroplast envelop: structure, function and role in leaf metabolism. *Annual Reviews of Plant Physiology* **32**, 139–168.
- Hendry GAF.** 1993. Evolutionary origins and natural functions of fructans—a climatological, biogeographic and mechanistic appraisal. *New Phytologist* **123**, 3–14.
- Hideg E, Barta C, Kalai T, Vass I, Hideg K, Asada K.** 2002. Detection of singlet oxygen and superoxide with fluorescent sensors in leaves under stress by photo inhibition or UV radiation. *Plant and Cell Physiology* **43**, 1154–1164.
- Hincha DK, Hellwege EM, Heyer AG, Crowe JH.** 2000. Plant fructans stabilize phosphatidylcholine liposomes during freeze-drying. *European Journal of Biochemistry* **267**, 535–540.
- Hincha DK, Zuther E, Hellwege EM, Heyer AG.** 2002. Specific effects of fructo- and gluco-oligosaccharides in the preservation of liposomes during drying. *Glycobiology* **12**, 103–110.
- Hincha DK, Zuther E, Heyer AG.** 2003. The preservation of liposomes by raffinose family oligosaccharides during drying is mediated by effects on fusion and lipid phase transitions. *Biochimica et Biophysica Acta* **1612**, 172–177.
- Hinrichs WLJ, Prinsen MG, Frijlink HW.** 2001. Inulin glasses for the stabilization of therapeutic proteins. *International Journal of Pharmacology* **215**, 163–174.
- Kaesler W.** 1983. Ultrastructure of storage cells in Jerusalem artichoke tubers (*Helianthus tuberosus* L.). Vesicle formation during inulin synthesis. *Zeitschrift für Pflanzenphysiologie* **111**, 253–260.
- Kang EMS.** 2007. Dietary flavonoids as protectors from ascorbate-induced oxidative stress *in vitro*. MSc dissertation, University of Saskatchewan, Saskatoon.
- Kardosova A, Ebringerova A, Alfoldi J, Nosalova G, Franova S, Hribalova V.** 2003. A biologically active fructan from the roots of *Arctium lappa* L., var. hercules. *International Journal of Biological Macromolecules* **33**, 135–140.
- Kawakami A, Sato Y, Yoshida M.** 2008. Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. *Journal of Experimental Botany* **59**, 803–814.
- Keller F, Pharr DM.** 1996. Metabolism of carbohydrates in sinks and sources: galactosyl-sucrose oligosaccharides. In: Zamski E, Schaffer AA, eds. *Photoassimilate distribution in plants and crops: source-sink relationships*. New York: Marcel Dekker, 157–183.
- Kellos T, Timar V, Szilagyi G, Szalai G, Galiba G, Kocsy G.** 2008. Stress hormones and abiotic stresses have different effects on antioxidants in maize lines with different sensitivity. *Plant Biology* **10**, 563–572.
- Klotke J, Kopka J, Gatzke N, Heyer AG.** 2004. Impact of soluble sugar concentrations on the acquisition of freezing tolerance in accessions of *Arabidopsis thaliana* with contrasting cold adaptation—evidence for a role of raffinose in cold acclimation. *Plant, Cell and Environment* **27**, 1395–1404.
- Konstantinova T, Parvanova D, Atanassov A, Djilianov D.** 2002. Freezing tolerant tobacco transformed to accumulate osmoprotectants. *Plant Science* **163**, 157–164.
- Korn M, Petersek S, Petermock H, Heyer AG, Hincha DK.** 2008. Heterosis in the freezing tolerance, and sugar and flavonoid contents of crosses between *Arabidopsis thaliana* accessions of widely varying freezing tolerance. *Plant, Cell and Environment* **31**, 313–327.
- Kristensen BK, Ammitzboll H, Rasmussen SK, Nielsen KA.** 2001. Transient expression of a vacuolar peroxidase increases susceptibility of epidermal barley cells to powdery mildew. *Molecular Plant Pathology* **2**, 311–317.
- Kytridis VP, Manetas Y.** 2006. Mesophyll versus epidermal anthocyanins as potential *in vivo* antioxidants: evidence linking the putative antioxidant role to the proximity of oxy-radical source. *Journal of Experimental Botany* **57**, 2203–2210.
- Le Roy K, Vergauwen R, Cammaer V, Yoshida M, Kawakami A, Van Laere A, Van den Ende W.** 2007. Fructan 1-exohydrolase is associated with flower opening in *Campanula rapunculoides*. *Functional Plant Biology* **34**, 972–983.
- Lehle L, Tanner W.** 1973. The function of myo-inositol in the biosynthesis of raffinose: purification and characterisation of galactinol:sucrose-6-galactosyltransferase from *Vicia faba* seeds. *European Journal of Biochemistry* **38**, 103–110.
- Lehner A, Bailly C, Flechel B, Poels P, Cume D, Corbineau F.** 2006. Changes in wheat seed germination ability, soluble carbohydrate and antioxidant enzyme activities in the embryo during the desiccation phase of maturation. *Journal of Cereal Science* **43**, 175–182.
- Li HJ, Yang AF, Zhang XC, Gao F, Zhang JR.** 2007. Improving freezing tolerance of transgenic tobacco expressing sucrose:sucrose 1-fructosyltransferase gene from *Lactuca sativa*. *Plant Cell, Tissue and Organ Culture* **89**, 37–48.

- Lineberger RD, Steponkus PL.** 1980. Cryoprotection by glucose, sucrose and raffinose to chloroplast thylakoids. *Plant Physiology* **65**, 298–304.
- Martinez-Fleites C, Ortiz-Lombardia M, Pons T, Tarbouriech N, Taylor EJ, Arrieta JG, Hernandez L, Davies GJ.** 2005. Crystal structure of levansucrase from the Gram-negative bacterium *Gluconacetobacter diazotrophicus*. *Biochemical Journal* **390**, 19–27.
- Mehlhorn H, Lelandais M, Korth HG, Foyer CH.** 1996. Ascorbate is the natural substrate for plant peroxidase. *FEBS Letters* **378**, 203–206.
- Mika A, Lúthje S.** 2003. Properties of guaiacol peroxidase activities isolated from corn root plasma membranes. *Plant Physiology* **132**, 1489–1498.
- Millar AH, Mittova V, Kiddle G, Heazlewood JL, Bartoli CG, Theodoulou FL, Foyer CH.** 2003. Control of ascorbate synthesis by respiration and its implications for stress responses. *Plant Physiology* **133**, 443–447.
- Mita S, Suzuki-Fujii K, Nakamura K.** 1995. Sugar-inducible expression of a gene for β -amylase in *Arabidopsis thaliana*. *Plant Physiology* **107**, 895–904.
- Mittler R.** 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* **7**, 405–410.
- Mittler R, Vanderauwera S, Gollery M, van Breusegem F.** 2004. Reactive oxygen gene network of plants. *Trends in Plant Science* **9**, 490–498.
- Møller IM, Jensen PE, Hansson A.** 2007. Oxidative modifications to cellular components in plants. *Annual Reviews of Plant Biology* **58**, 459–481.
- Morelli R, Russo-Volpe S, Bruno N, Scalzo RL.** 2003. Fenton-dependent damage to carbohydrates: free radical scavenging activity of some simple sugars. *Journal of Agricultural and Food Chemistry* **51**, 7418–7425.
- Morsy MR, Jouve L, Hausman JF, Hoffmann L, McD Stewart J.** 2007. Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *Journal of Plant Physiology* **164**, 157–167.
- Morvan-Bertrand A, Boucaud J, Le Saos J, Prud'homme MP.** 2001. Roles of the fructans from the leaf sheaths and from the elongating leaf bases in the regrowth following defoliation of *Lolium perenne* L. *Planta* **213**, 109–120.
- Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI.** 2007. Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta* **1767**, 414–421.
- Nery DCM, da Silva CG, Mariani D, Fernandes PN, Pereira MD, Panek AD, Eleutherio ECA.** 2008. The role of trehalose and its transporter in protection against reactive oxygen species. *Biochimica et Biophysica Acta* **1780**, 1408–1411.
- Nishikawa N, Kato M, Hyodo H, Ikoma Y, Sugiura M, Yano M.** 2005. Effect of sucrose on ascorbate level and expression of genes involved in the ascorbate biosynthesis and recycling pathway in harvested broccoli florets. *Journal of Experimental Botany* **56**, 65–72.
- Nishiyama Y, Allakhverdiev SI, Murata N.** 2006. A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochimica et Biophysica Acta* **1757**, 742–749.
- Nishizawa A, Yukinori Y, Shigeoka S.** 2008. Galactinol and raffinose as a novel function to protect plants from oxidative damage. *Plant Physiology* **147**, 1251–1263.
- Orozco-Cardenas ML, Narváez-Vásquez J, Ryan CA.** 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *The Plant Cell* **13**, 179–191.
- Panikulangara TJ, Eggers-Schumacher G, Wunderlich M, Stransky H, Schöffl F.** 2004. Galactinol synthase1: a novel heat shock factor target gene responsible for heat-induced synthesis of raffinose family oligosaccharides in Arabidopsis. *Plant Physiology* **136**, 3148–3158.
- Paradiso A, Cecchini C, De Gara L, D'Egidio MG.** 2006. Functional, antioxidant and rheological properties of meal from immature durum wheat. *Journal of Cereal Science* **43**, 216–222.
- Parvanova D, Ivanov S, Konstantinova T, Karanov E, Atanasov A, Tsvetkov T, Alexieva V, Djilianov D.** 2004. Transgenic tobacco plants accumulating osmolytes show reduced oxidative damage under freezing stress. *Plant Physiology and Biochemistry* **42**, 57–63.
- Passardi F, Penel C, Dunand C.** 2004. Performing the paradoxical: how plant peroxidases modify the cell wall. *Trends in Plant Science* **9**, 534–540.
- Peterbauer T, Puschenreiter M, Richter A.** 1998. Metabolism of galactosylononitol in seeds of *Vigna umbellata*. *Plant and Cell Physiology* **39**, 334–341.
- Pignocchi C, Foyer CH.** 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. *Current Opinion in Plant Biology* **6**, 379–389.
- Pourcel L, Routaboul JM, Cheynier V, Lepiniec L, Debeaujon I.** 2007. Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends in Plant Science* **12**, 29–36.
- Price J, Laxmi A, Martin SKS, Jang JC.** 2004. Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. *The Plant Cell* **16**, 2128–2150.
- Reisen D, Leborgne-Castel N, Ozalp C, Chaumont F, Marty F.** 2003. Expression of a cauliflower tonoplast aquaporin tagged with GFP in tobacco suspension cells correlates with an increase in cell size. *Plant Molecular Biology* **52**, 387–400.
- Rolland F, Gonzalez EB, Sheen J.** 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Reviews of Plant Biology* **57**, 676–709.
- Romero-Puertas MC, Rodríguez-Serrano M, Corpas FJ, Gómez M, Delrío LA, Sandalio LM.** 2004. Cadmium-induced subcellular accumulation of O_2^- and H_2O_2 in pea leaves. *Plant, Cell and Environment* **27**, 1122–1134.
- Santarius KA, Milde H.** 1977. Sugar compartmentation in frost-hardy and partially dehardened cabbage leaf cells. *Planta* **136**, 163–166.
- Santarius KA.** 1973. The protective effect of sugars on chloroplast membranes during temperature and water stress and its relationship to frost, desiccation, and heat resistance. *Planta* **113**, 105–114.
- Sato Y, Murakami T, Funatsuki H, Matsuba S, Saruyama H, Tanida M.** 2001. Heat shock-mediated APX gene expression and protection against chilling injury in rice seedlings. *Journal of Experimental Botany* **52**, 145–151.
- Scarpeci TE, Valle EM.** 2008. Rearrangement of carbon metabolism in *Arabidopsis thaliana* subjected to oxidative stress condition: an emergency survival strategy. *Plant Growth Regulation* **54**, 133–142.
- Scott I, Logan DC.** 2008. Mitochondrial morphology transition is an early indicator of subsequent cell death in Arabidopsis. *New Phytologist* **177**, 90–101.
- Shi QH, Wang XF, Wei M.** 2007. Nitric oxide modulates the metabolism of plasma membrane and tonoplast in cucumber roots. *Acta Horticulturae* **761**, 275–282.
- Slesak I, Libik M, Miszalski Z.** 2008. The foliar concentration of hydrogen peroxide during salt-induced C3–CAM transition in *Mesembryanthemum crystallinum* L. *Plant Science* **174**, 221–226.

- Smirnoff N, Cumbes QJ.** 1989. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* **28**, 1057–1060.
- Sottomayor M, Ros Barceló A.** 2003. Peroxidase from *Catharanthus roseus* (L.) G. Don and the biosynthesis of a-3',4'-anhydrovinblastine: a specific role for a multifunctional enzyme. *Protoplasma* **222**, 97–105.
- Sottomayor M, Cardoso IL, Pereira LG, Ros Barceló A.** 2004. Peroxidase and the biosynthesis of terpenoid indole alkaloids in the medicinal plant *Catharanthus roseus* (L.) G. Don. *Phytochemistry Reviews* **3**, 159–171.
- Sulmon C, Gouesbet G, El Amrani A, Couée I.** 2006. Sugar-induced tolerance to the herbicide atrazine in *Arabidopsis* seedlings involves activation of oxidative and xenobiotic stress responses. *Plant Cell Reports* **25**, 489–498.
- Surpin M, Raikhel NV.** 2004. Traffic jams affect plant development and signal transduction. *Nature Reviews Molecular Cell Biology* **5**, 100–109.
- Suzuki N, Mittler R.** 2006. Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. *Physiologia Plantarum* **126**, 45–51.
- Takahama U.** 2004. Oxidation of vacuolar and apoplastic phenolics substrates by peroxidase: physiological significance of the oxidation reactions. *Phytochemistry Reviews* **3**, 207–219.
- Takahashi S, Murata N.** 2008. How do environmental stresses accelerate photoinhibition? *Trends in Plant Science* **13**, 178–182.
- Tapernoux-Lüthi EM, Böhm A, Keller F.** 2004. Cloning, functional expression, and characterization of the raffinose oligosaccharide chain elongation enzyme, galactan:galactan galactosyltransferase, from common bugle leaves. *Plant Physiology* **134**, 1377–1387.
- Uemura M, Steponkus PL.** 2003. Modification of the intracellular sugar content alters the incidence of freeze-induced membrane lesions of protoplasts isolated from *Arabidopsis thaliana* leaves. *Plant, Cell and Environment* **26**, 1083–1096.
- Valluru R, Lammens W, Claupein W, Van den Ende W.** 2008. Freezing tolerance by vesicle-mediated fructan transport. *Trends in Plant Science* **13**, 409–414.
- Valluru R, Van den Ende W.** 2008. Plant fructans in stress environments: emerging concepts and future prospects. *Journal of Experimental Botany* **59**, 2905–2916.
- Van den Ende W, De Coninck B, Van Laere A.** 2004. Plant fructan exohydrolases: a role in signaling and defense? *Trends in Plant Science* **9**, 523–528.
- Van den Ende W, Michiels A, Van Wouterghem D, Vergauwen R, Van Laere A.** 2000. Cloning, developmental, and tissue-specific expression of sucrose:sucrose 1-fructosyl transferase from *Taraxacum officinale*. Fructan localization in roots. *Plant Physiology* **123**, 71–79.
- Van den Ende W, Van Laere A.** 1996. *De-novo* synthesis of fructans from sucrose *in vitro* by a combination of two purified enzymes (sucrose:sucrose 1-fructosyltransferase and fructan:fructan 1-fructosyltransferase) from chicory roots (*Cichorium intybus* L.). *Planta* **200**, 335–342.
- Van den Ende W, Yoshida M, Clerens S, Vergauwen R, Kawakami M.** 2005. Cloning, characterization and functional analysis of novel 6-kestose exohydrolases (6-KEHs) from wheat (*Triticum aestivum* L.). *New Phytologist* **166**, 917–932.
- Van Laere A, Van den Ende W.** 2002. Inulin metabolism in dicots: chicory as a model system. *Plant, Cell and Environment* **25**, 803–813.
- Van Riet L, Altenbach D, Vergauwen R, Clerens S, Kawakami A, Yoshida M, Van den Ende W, Wiemken A, Van Laere A.** 2008. Purification, cloning and functional differences of a third fructan 1-exohydrolase (1-FEHw3) from wheat (*Triticum aestivum*). *Physiologia Plantarum* **133**, 242–253.
- Vereyken IJ, Albert van Kuik J, Evers TH, Rijken PJ, de Kruijff B.** 2003. Structural requirements of the fructan–lipid interaction. *Biophysical Journal* **84**, 3147–3154.
- Wang N, Nobel PS.** 1998. Phloem transport of fructans in the crassulacean acid metabolism species *Agave deserti*. *Plant Physiology* **116**, 709–714.
- Wang P, Song CP.** 2008. Guard-cell signalling for hydrogen peroxide and abscisic acid. *New Phytologist* **178**, 703–718.
- Whiteman SA, Nühse TS, Ashford DA, Sanders D, Maathuis FJM.** 2008. A proteomic and phosphoproteomic analysis of *Oryza sativa* plasma membrane and vacuolar membrane. *The Plant Journal* **56**, 146–156.
- Wubbolts R, Fernandez-Borja M, Oomen L, Verwoerd D, Janssen H, Calafat J, Tulp A, Dusseljee S, Neeffjes J.** 1996. Direct vesicular transport of MHC class II molecules from lysosomal structures to the cell surface. *Journal of Cell Biology* **135**, 611–622.
- Xiong Y, Contento AL, Nguyen PQ, Bassham DC.** 2007. Degradation of oxidized proteins by autophagy during oxidative stress in *Arabidopsis*. *Plant Physiology* **143**, 291–299.
- Yamasaki H, Grace SC.** 1998. EPR detection of phytophenoxyl radicals stabilized by zinc ions: evidence for the redox coupling of plant phenolics with ascorbate in the H₂O₂-peroxidase system. *FEBS Letters* **422**, 377–380.
- Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W.** 2004. GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiology* **136**, 2621–2632.
- Zuther E, Buchel K, Hundertmark M, Stitt M, Hinch DK, Heyer AG.** 2004. The role of raffinose in the cold acclimation response of *Arabidopsis thaliana*. *FEBS Letters* **576**, 169–173.