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— REVIEW ARTICLE —

Plant invertases: structure, function and regulation of a diverse enzyme family

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Invertase is a key metabolic enzyme which hydrolyzes the disaccharide sucrose (the major type of sugar transported through the phloem of higher plants) to glucose and fructose. In higher plants, invertase exists in several isoforms with different biochemical properties and subcellular locations. The specific functions of the different invertase isoforms are not clear, but they appear to regulate the entry of sucrose into the different utilization pathways. Invertases, alone or in combination with plant hormones, are involved in regulating developmental processes, carbohydrate partitioning, as well as biotic and abiotic interactions. The current knowledge about this isoenzyme family, with special reference to recent key findings, is reviewed here.

Key words: invertase, sugar metabolism, gene expression, regulation.

INTRODUCTION

Plant invertases (b-fructofuranosidase, EC 3.2.1.26) comprise a family of enzymes which catalyze the hydrolysis of sucrose to glucose and fructose. Higher plants contain several invertase isoenzymes, which can be distinguished by their subcellular localization (cell wall, vacuole or cytosol), solubility (soluble or insoluble in low ionic strength buffer), optimum pH (acid or neutral/alkaline) and isoelectric point (p*I*) (Sturm & Chrispeels, 1990). Cell wall and vacuolar invertases are glycosylated forms with an acid pH optimum, while cytosolic invertase is most likely a non-glycosylated form with a neutral/alkaline optimum pH. Glycosylation of acid invertases is required for their transport across either the plasma membrane or the tonoplast, hence their localization in the cell wall or the vacuole (Tymowska-Lalanne & Kreis, 1998a). Cell wall invertases are ionically bound to the cell wall via positive charges due to their neutral/basic p*I* at low pH (Kim *et al*., 2000). However, Carlson & Chourey (1999) have demonstrated the presence of contaminating cell wall invertase in the soluble fraction of developing maize kernels, suggesting that these ionic bonds are rather weak. The main characteristics of the three types of plant invertases are summarized in Table 1.

Table 1. Properties of plant invertases

Type of invertase	pH optimum	Solubility	Glycosylation
Cell wall (acid)	4.5 - 5.0	Insoluble	+
Vacuolar (acid)	4.5 - 5.0	Soluble	+
Cytoplasmic (alkaline)	7.0 - 7.8	Soluble	–

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ENZYMATIC PROPERTIES OF PLANT INVERTASES

Vacuolar and cell wall invertases share some biochemical properties, e.g. they cleave sucrose most efficiently between pH 4.5 and 5.0 and attack the disaccharide from the fructose residue. Both types of invertases are β-fructofuranosidases and also hydrolyze other fructofuranosides such as raffinose and stachyose but with significantly reduced cleavage efficiency. Contrary to the acid invertases, neutral and alkaline invertases appear to be sucrose specific (Sturm, 1999).

Acid invertases have a *K*m for sucrose in the low mM range. Activity is inhibited by heavy metal ions such as Hg^{2+} and Ag^{+}, suggesting the presence of a sulfhydryl group at the catalytic site. Acid invertases are also inhibited by their reaction products, with glucose acting as a non-competitive inhibitor and fructose as a competitive inhibitor. The majority of the mature polypeptides have molecular masses between 55 and 70 kD. In the case of cytosolic invertases, most native polypeptides are homotetramers composed of subunits with a molecular mass of 54 to 65 kD. The polypeptides preferentially hydrolyze sucrose with a *K*m of 10 mM. They are strongly inhibited by glucose and fructose, but not by heavy metal ions, suggesting marked differences between the catalytic sites of neutral/alkaline and acid invertases. It should be noted that cytosolic invertases are extremely labile and enzyme activity is rapidly lost after tissue homogenization, thus rendering their purification very difficult.

Analysis of some of the purified proteins on denaturing SDS gels under reducing conditions revealed the presence of proteolytic fragments (for review, Sturm, 1999). Under native conditions, these fragments appear to be tightly associated and, in a complex, possess enzymatic activity. Fragmentation does not appear to be an artifact of protein purification, but instead seems to be under developmental control. For example, Arai *et al*. (1991) have demonstrated that the full-length proteins predominate in very young hypocotyls of mung bean, whereas with increasing hypocotyl age the N- and C-terminal fragments were more abundant. Their functional importance however is not yet fully understood.

MOLECULAR PROPERTIES AND GENE STRUCTURE OF PLANT INVERTASES

Many genes and cDNAs coding cell wall (acid insoluble) and vacuolar (acid soluble) invertases have been cloned from various plant species (for review, Tymowska-Lalanne & Kreis, 1998a; Goetz & Roitsch, 2000). In comparison, only a few cDNAs or genes encoding neutral/alkaline cytosolic invertases have been reported in plants (Gallagher & Pollock, 1998; Sturm *et al*., 1999). According to Tymowska-Lalanne & Kreis (1998a), cell wall and vacuolar invertases are synthesized as pre-proproteins, with a long leader sequence which is cleaved off during transport and protein maturation (Fig. 1). This leader sequence most likely consists of a signal peptide, required for entry into the endoplasmic reticulum and, thus, into the secretory pathway (Blobel, 1980), and an N-terminal propeptide, thought to either play a role in protein folding and stability (Klionsky *et al*., 1988) and/or in the regulation of enzyme activity (Hasilik & Tanner, 1987). Contrary to the cell wall enzyme, the vacuolar proteins contain a short hydrophobic C-terminal extension, which might be involved in the vacuolar targeting of the protein (Unger *et al*., 1994).

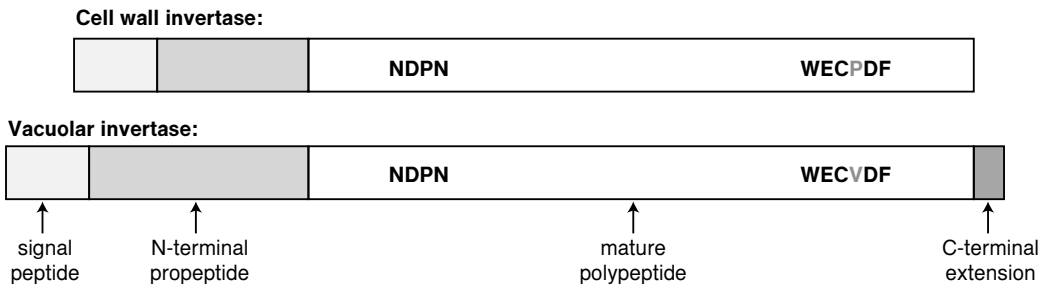

FIG. 1. Schematic representation of cell wall and vacuolar invertases. The peptide sequences NDPN and $WEC^{V}/_{P}DF$ represent the β-fructosidase motif and the catalytic site, respectively. Diagram adapted from Sturm (1999).

In general, plant invertase genes have a fairly similar structure and contain six to eight exons (Sturm, 1999). With the exception of a cloned gene encoding a cell wall-bound invertase in carrot (*InvDC1*; Ramloch-Lorenz *et al*., 1993), all other invertase genes contain one conserved exon (exon 2), which is only 9 bp long (Tymowska-Lalanne & Kreis, 1998a). This exon is the smallest functional exon known in the plant kingdom (Kim *et al*., 2000) and encodes three amino acids (DPN) which are part of the highly conserved β-fructosidase motif NDPN (Goetz & Roitsch, 2000). This motif together with the well-conserved cysteine catalytic site (WEC^{V}/$_{P}$DF) might have an important function in enzyme

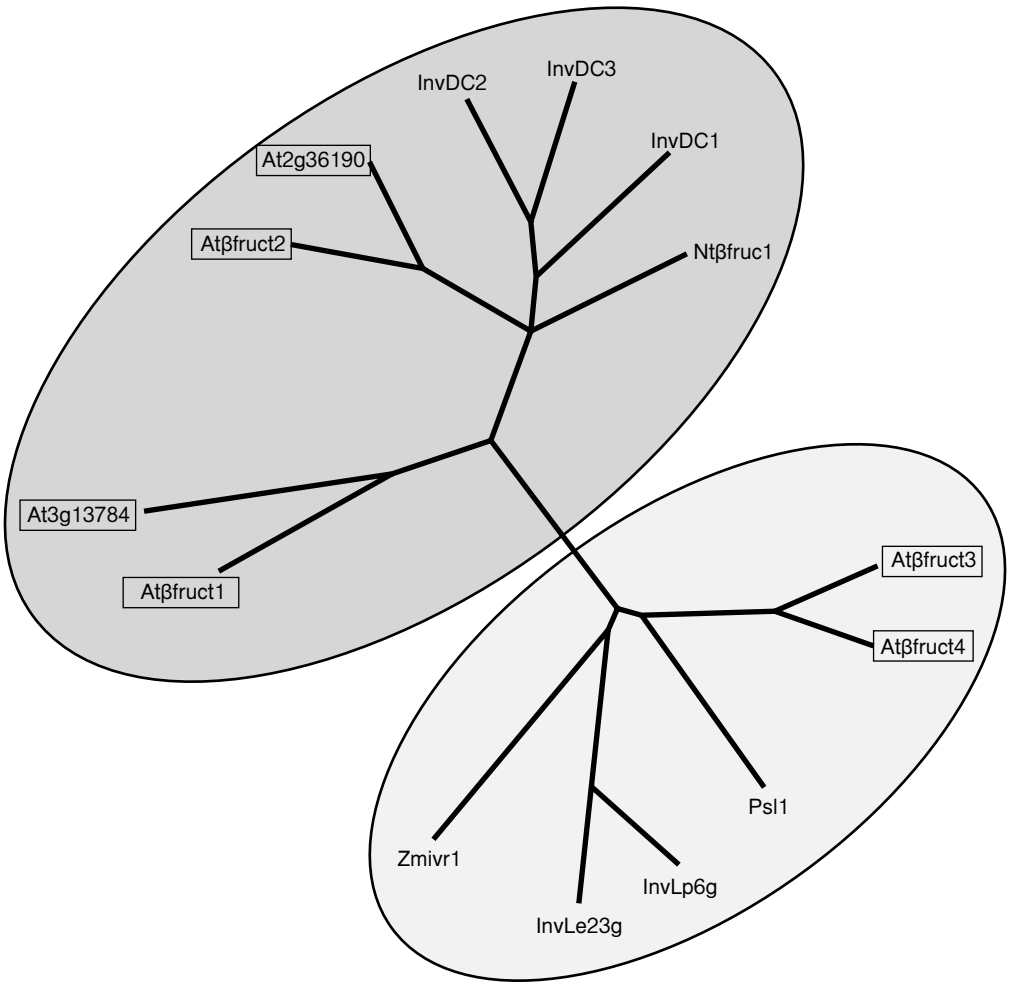

FIG. 2. Phylogenetic relationships of different, characterized and putative, plant invertases. The dendrogram shown was generated using the DRAWTREE program of the PHYLIP package (Felsenstein, 1989) by comparison of the deduced amino acid sequences of plant invertases. Light oval indicates vacuolar invertase isoforms, whereas dark oval indicates cell wall invertase isoforms. *Arabidopsis* invertase isoforms are boxed. Gene names and EMBL accession numbers: *Arabidopsis thaliana*: *Atbfruct1*, X74515 (Schwebel-Dugué *et al*., 1994); *Atbfruct2*, U11033 (Mercier & Gogarten, 1995); *Atbfruct3*, X99111 (Haouazine-Takvorian *et al*., 1997); *Atbfruct4*, Y11559 (Haouazine-Takvorian *et al*., 1997); carrot (*Daucus carota*): *InvDC1*, X69321 (Ramloch-Lorenz *et al*., 1993); *InvDC2*, X78424 (Lorenz *et al*., 1995); *InvDC3*, X78423 (Lorenz *et al*., 1995); tomato (*Lycopersicon esculentum*): *InvLe23g*, Z12027 (Elliott *et al*., 1993); tomato (*Lycopersicon pimpinellifolium*): *InvLp6g*, Z12028 (Elliott *et al*., 1993); maize (*Zea mays*): *Zmivr1*, U16123 (Xu *et al*., 1995); Tobacco (*Nicotiana tabacum*): *Ntbfruc1*, X81834 (Greiner *et al*., 1995); pea (*Pisum sativum*): PsI1, AY112702 (Zhang, 2002). Other genes (*At2g36190, At3g13784*) represent uncharacterized, putative *Arabidopsis* invertase isoforms, as determined by MIPS database (M*At*DB) searches and sequence analysis. (It should be noted that the putative *Arabidopsis* cell wall invertase genes *At1g55120* and *At5g11920* were not included in the tree, because a research paper just published by De Coninck *et al*. (2005) has shown that *At1g55120* and *At5g11920* show in fact fructan exohydrolase activity, as demonstrated by heterologous expression in *Pichia pastoris*).

conformation or catalytic activity.

Detailed amino acid analysis has identified that cell wall-bound invertases have a proline residue in the cysteine catalytic site, while vacuolar invertases have a valine residue (Tymowska-Lalanne & Kreis, 1998a). Goetz & Roitsch (2000) have also demonstrated that the aspartate (D) from the NDPN box and the glutamate (E) and cysteine (C) from the $WEC^{V}/_{P}DF$ box are essential for enzyme activity, by introducing specific amino acid substitutions by site-directed mutagenesis and heterologously expressing the mutated genes in an invertase deficient *Saccharomyces cerevisiae* strain.

The comparison of the deduced amino acid sequences for cell wall and vacuolar invertases has also demonstrated that the two forms belong to two different classes (Tymowska-Lalanne & Kreis, 1998a). A higher similarity is seen between members of the same form (cell wall or vacuolar) from different species compared with different forms from the same species (Tymowska-Lalanne & Kreis, 1998a). For example, the *Arabidopsis thaliana* cell wall invertases are more closely related to the carrot cell wall invertases than to the *Arabidopsis* vacuolar invertases (Fig. 2). This implies that perhaps invertase gene duplication and divergence occurred prior to the separation of these plant species during evolution (Tymowska-Lalanne & Kreis, 1998a). Interestingly, in addition to the two major classes being identified during sequence analysis of plant invertase genes, multiple isoenzymes or isoforms of invertase have been shown to exist in the same plant tissue (Weber *et al*., 1995; Godt & Roitsch, 1997; Sherson *et al*., 2003).

PHYSIOLOGICAL ROLE AND EXPRESSION OF PLANT INVERTASES

The physiological functions of invertase isoforms are complex and depend upon the kind of tissue and the subcellular location (Sturm & Tang, 1999; Tang *et al*., 1999; Roitsch & González, 2004) (Fig. 3). The acid soluble (vacuolar) invertases not only mobilize sucrose and/or control sugar composition in vacuoles but they also play a role in establishing sink (area of growth or storage) tissue in storage organs, such as in the mature tomato fruit (Klann *et al*., 1993). Vacuolar invertases may also play a role in maintaining cell turgor and cell expansion (Sebkova *et al*., 1995). Tissues undergoing rapid cell expansion usually have a high hexose and low sucrose concentration suggesting an important role for sucrose-metabolizing enzymes (Sebkova *et al*., 1995). More recently, Mitsuhashi *et al*. (2004) have detected high transcript levels of the vacuolar invertases *Atβfruct3*

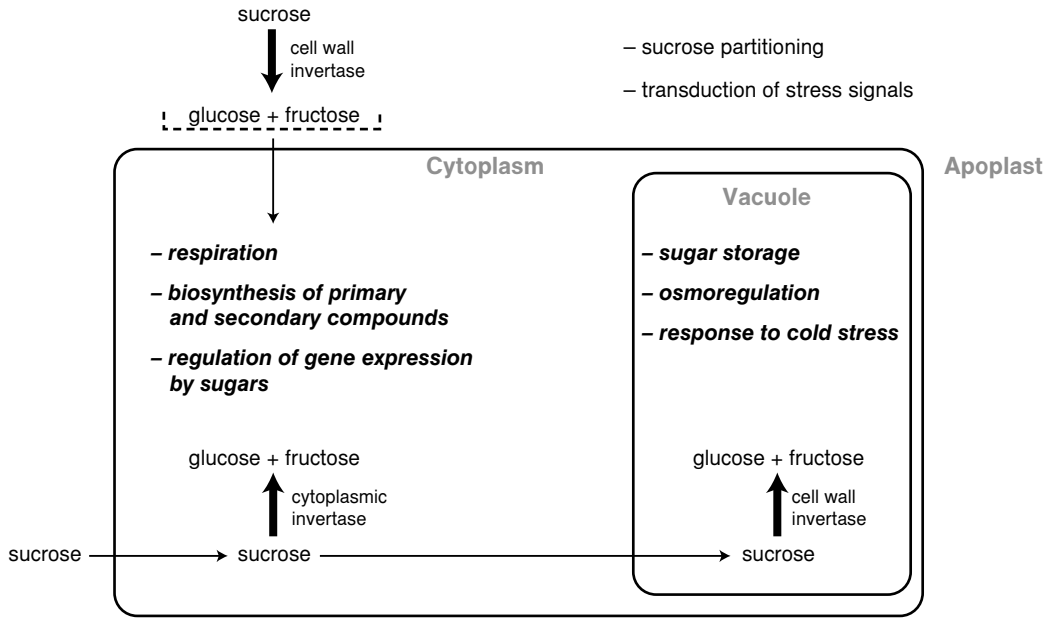

FIG. 3. Subcellular locations and proposed functions of plant invertases. Plant cells contain different invertase isoforms in the apoplast, cytoplasm and vacuole. Hexoses generated by the activities of the different enzymes have different functions (a few important examples are indicated in italics). Diagram adapted from Sturm (1999).

and *Atβfruct4* in germinating *A. thaliana* seeds, suggesting that they may trigger the expansion of the embryo. Acid insoluble (cell wall-bound) invertases are considered to be key enzymes in sucrose unloading and in the source/sink balance within the plant (Godt & Roitsch, 1997; Tang *et al*., 1999), by supplying carbohydrates to sink tissues *via* the apoplastic pathway. Neutral/alkaline invertases are most probably located in the cytosol, as is sucrose synthase, another sucrose-cleaving enzyme. The cytoplasmic invertase is most likely involved in the regulation of intracellular glucose and fructose levels in mature tissues in which sucrose synthase activity is low (Van den Ende & Van Laere, 1995), providing the cell with energy for metabolic processes (Sturm & Tang, 1999).

In addition to the complex functions of the invertases, it also appears that different invertase isoforms may play a variety of roles during the phases of cell development. Tang *et al*. (1999) have conducted one of the most detailed investigations on the functions of cell wall and vacuolar invertases. Using antisense techniques, they obtained transgenic carrot plants with reduced expression of the cell wall invertase *InvDC1* (Sturm & Chrispeels, 1990) and the vacuolar invertase *sI* (Unger *et al*., 1994). Investigations have shown that both cell wall and vacuolar antisense plants were profoundly affected at early stages of development, with both plant types being severely stunted (Tang *et al*., 1999). When the seedlings were supplemented with glucose and fructose in the growing medium, they showed complete recovery and looked normal. At later stages of development however, antisense plants were affected differently. Plants expressing antisense mRNA for cell wall invertase appeared bushy due to the development of extra leaves (which accumulated elevated levels of sucrose and starch) while the tap root exhibited reduced development, with lower carbohydrate content (Tang *et al*., 1999). Vacuolar antisense plants had also more leaves than control plants, although tap roots developed normally (Tang *et al*., 1999). It appears that acid invertases play an important role in early plant development, most likely *via* control of sugar composition and metabolic fluxes (Tang *et al*., 1999). Later in plant development, both isoenzymes seem to have important functions in sucrose partitioning.

Further evidence that the expression of invertase genes is developmentally regulated has been provided for a number of plants including *Arabidopsis*, bean and tomato. Tymowska-Lalanne & Kreis (1998b) have demonstrated that the *Arabidopsis* vacuolar invertase *Atβfruct4* is expressed in the leaves of very young plants but no transcripts were detected in the leaves of mature plants, while Blee and Anderson (2002) have reported that the accumulation of transcripts for the bean vacuolar invertase *Pvsai* was age-dependent in the roots, showing peak mRNA levels by 3 days but declining by 7 days after planting. Similarly, Elliott *et al*. (1993) have investigated the role of vacuolar invertase during the ripening of tomato fruit in two different species, *Lycopersicon esculentum* and *L. pimpinellifolium*. In *L. pimpinellifolium*, vacuolar invertase was expressed in the green fruit stage, increasing afterwards until a peak was reached in the ripe fruits. In *L. esculentum*, vacuolar invertase expression was observed in the intermediate fruits and peaked in ripe fruits, with no signal detected at the green stage of fruit development.

Interestingly, several invertase isoforms also appear to be expressed in an organ-specific manner. For example, Tymowska-Lalanne & Kreis (1998b) have carried out RT-PCR analyses and have demonstrated that the expression of the *Arabidopsis* cell wall invertase *Atβfruct2* is flower-specific, although more recent studies by Sherson *et al*. (2003) have demonstrated that low levels of *Atβfruct2* transcripts were also detected in seeds, while the putative cell wall invertase *At3g13784* appears to be floral-specific. Similar RT-PCR studies using total RNA extracted from maize plants have shown that *Incw3*, a vacuolar invertase, is expressed in most reproductive organs, except for ovaries, and in the vegetative organs such as roots and young stems, but not in young and mature leaves (Kim *et al*., 2000). Roitsch *et al*. (1995) have reported a sink-specific distribution of the activity and mRNA levels of a cell wall invertase from *Chenopodium rubrum*. The transcript of this gene could be detected only in roots and not in mature leaves and stems.

FACTORS AFFECTING INVERTASE EXPRESSION

Plant invertase gene expression and enzyme activity are both known to be influenced by a variety of intracellular and extracellular factors. These factors modulate invertase activity either by activation or by repression, acting either at the level of gene expression and/or at the level of protein activity (Tymowska-Lalanne & Kreis, 1998a). The following section briefly reviews these factors.

Sugars

Sugars, known to act as signaling molecules regulating a variety of genes in different physiological pathways (Koch, 1996; Sheen *et al*., 1999), also modulate the activity of invertase, although the mechanisms of the signaling pathways remain unclear. Burch *et al*. (1992) have shown that the activity of vacuolar invertase from potato leaves and tubers is inhibited in a competitive manner by fructose and in a non-competitive manner by glucose. This confirms the observation by Isla *et al*. (1991) about the competitive inhibition of potato tuber invertase by fructose, while Lopez *et al*. (1988) have shown that acid invertase from *Carica papaya* fruits was inhibited by fructose but not by glucose. Cho *et al*. (2005) have recently demonstrated that the expression of the acid invertase *OsCIN5* in excised rice leaves was significantly suppressed when leaves were treated with sucrose.

In contrast to the above reports about direct inhibition of invertase enzyme activity by sugars, several authors have demonstrated the stimulation of invertase enzyme levels by sugars. The increase in enzyme activity of cell wall invertase by glucose in *C. rubrum* was shown to run parallel to the increased level of mRNA of *CIN1* (Roitsch *et al*., 1995). Expression of a cell wall invertase isoform has also been shown to be induced by glucose in tobacco (Krausgrill *et al*., 1996), *Arabidopsis* (Tymowska-Lalanne & Kreis, 1998b) and tomato (Sinha *et al*., 2002). However, other studies have shown that sugars do not modify invertase activity in various cell types or organs (Sturm & Chrispeels, 1990; Weil & Rausch, 1990). Although contradictory, these results might simply stem from the fact that isoenzymes, which were extracted from different organs, were either repressed or enhanced by sugar availability (Tymowska-Lalanne & Kreis, 1998a). Results obtained by Xu *et al*. (1996) indicate that this is probably the case. Their data showed that the expression of two vacuolar invertase genes from maize is sugar-regulated, involving different patterns of sugar induction and repression. One maize gene (*Ivr1*) encoding vacuolar invertase is repressed by the presence of sugars and up-regulated by their depletion, whereas a second gene (*Ivr2*) is up-regulated by increasing sugar supply. Similar induction of *Ivr2* by glucose in maize leaves has also been reported in a more recent study by Trouverie *et al*. (2004).

Plant growth regulators

Plant growth regulators (PGRs) play an integral role in controlling growth, differentiation and development of plants. There is accumulating evidence that invertases are regulated by various PGRs which, in most cases, can be related to the increased carbohydrate demand of growth-stimulated tissues (Roitsch *et al*., 2003). For example, gibberellic acid (GA_3) has been reported to increase invertase activity in several plant organs (for review, Tymowska-Lalanne & Kreis, 1998a). Invertase mRNA from shoots of dwarf pea plants (*Pisum sativum*) was induced after GA_3 treatment, indicating that the expression of the shoot cell wall invertase gene could be regulated by GA_3 at transcriptional and/or translational levels (Wu *et al*., 1993). Similarly, Mitsuhashi *et al*. (2004) have shown an induction of both vacuolar and cell wall invertase genes in *Arabidopsis* seeds following treatment with GA_4, an active gibberellin.

It has been reported that the activity of cell wall invertase is also stimulated by auxin (Weil & Rausch, 1990). Morris & Arthur (1984) have observed that during cell expansion in bean stems, the highest level of invertase activity occurs simultaneously with the peak of indolyl-3-acetic acid concentration, while the exogenous application of indolyl-3-acetic acid promoted an increase in vacuolar invertase activity. Similarly, there is preliminary evidence for the significance of the induction of cell wall invertase by abscisic acid obtained with transgenic tobacco plants expressing a fusion between the promoter of the tomato cell wall invertase *Lin6* and the β-glucuronidase reporter gene (Roitsch *et al*., 2003). Cytokinins have also been implicated in affecting invertase expression. Tissues with elevated activities of cell wall invertase, such as rapidly growing tissues, are known to contain elevated cytokinin concentrations (Godt & Roitsch, 1997), while studies using autotrophic cell cultures showed that the cell wall invertases *CIN1* from *Chenopodium rubrum* (Ehness & Roitsch, 1997) and *Lin6* from tomato (Godt & Roitsch, 1997) are highly up-regulated in response to physiological concentrations of different cytokinins.

Abiotic stress

A variety of abiotic stress factors such as low temperature, oxygen deficiency, wounding, drought and salinity have been considered to affect the level of invertase activity (for reviews, Tymowska-Lalanne & Kreis, 1998a; Roitsch *et al*., 2003; Roitsch & Gon-

zález, 2004). Zhou *et al*. (1994) have detected vacuolar invertase transcripts in potato tubers stored at 1°C, but not in those stored at 10°C, while Zeng *et al*. (1999) showed that low oxygen stress lead to a decrease in expression of the vacuolar invertase isoforms *Ivr1* and *Ivr2* in maize root tips. Such a response reveals an important implication in acclimation to low oxygen stress by conserving sucrose and ATP and reducing the hexose-based sugar-signaling system (Zeng *et al*., 1999).

Wounding constitutes one of the most dramatic forms of environmental (abiotic) stress (Ehness *et al*., 1997). When plant tissues become damaged, (e.g. mechanical wounding), plant defense mechanisms are elicited, since plant responses to injury and wounding are very similar to those against pathogen attacks (Isaac, 1996). However, activation of defense reactions requires energy and thus induction of the sink metabolism (Ehness *et al*., 1997). Such increased demand in carbohydrate supply could potentially be met by the localized up-regulation of invertase in response to the stress-related stimulus (Roitsch *et al*., 2003). Evidence supporting this theory included the studies by Sturm & Chrispeels (1990) and Ehness *et al*. (1997), who showed that the mRNAs for the cell wall invertases *InvDC1* of carrot and *CIN1* of *C. rubrum* are induced in response to wounding.

Inhibitors

The low and variable invertase activity might be attributed, at least in part, to the presence of endogenous inhibitors (Greiner *et al*., 1998). Several invertase inhibitor proteins have been isolated and purified from a number of plant species (for review, Tymowska-Lalanne & Kreis, 1998a; Rausch & Greiner, 2004), and cDNA clones coding for cell wall and/or vacuolar invertase inhibitors have now been isolated and analyzed from tobacco (Greiner *et al*., 1998, 1999), *Arabidopsis* (Link *et al*., 2004), and maize (Bate *et al*., 2004). Interestingly, fairly low concentrations of sucrose prevent an inhibitor purified from a suspension culture of tobacco binding to the enzyme (Weil *et al*., 1994), thus raising questions about its postulated function. The deduced amino acid sequence of *NtCIF* shows homology to several sequences identified in the genomes of other plant species, including 15 genes in *Arabidopsis* (Sherson *et al*., 2003). A recombinant *Nt-inh1*-encoded protein was shown to inhibit the activities of cell wall invertases from tobacco and *C. rubrum* and vacuolar invertase from tomato *in vitro*, but no inhibitory activity was found towards two yeast invertases (Greiner *et al*., 1998), suggesting that an inhibitory effect on fungal pathogen invertases is rather unlikely (Greiner *et al*., 1998).

The physiological role of invertase inhibitors during plant development is not yet fully understood, however it has been hypothesized that the invertase inhibitor may operate as a regulatory switch for cell wall invertase, with the inhibitor always being bound to the cell wall invertase, but inducing the inhibitory conformational change only when sucrose concentration decreases below a certain threshold (Weil *et al*., 1994).

Pathogen infection

Of particular interest is the potential effect of pathogen infection on invertase activity. There is a growing body of evidence supporting the concept that infection by plant pathogens leads to increased levels of invertase activity, both acid and alkaline (for review, Hall & Williams, 2000). The majority of such reports refer to acid invertase activity (Storr & Hall, 1992; Clark & Hall, 1998; Chou *et al*., 2000), although increases in alkaline invertase have also been noted (Storr & Hall, 1992). An interesting point is that increases in invertase activity could be (partly) attributed to the potential presence of fungal invertase isoforms as well. Previous reports have identified invertase isoforms from biotrophic pathogenic fungi (Chou *et al*., 2000), which, if located peripherally in the fungal tissue or secreted into the surrounding medium, would further contribute to the invertase activity already present within the host (Mendgen & Hahn, 2002). In any case, it is not clear whether as a result of this increase in invertase activity in the infected tissues, the observed increased hexose levels act to support the defense responses or serve to provide a supply of hexoses for the growing pathogen (Fotopoulos *et al*., 2003).

Molecular investigations of expression have also provided evidence to support the observed increases in invertase activity following pathogen attack. Sturm & Chrispeels (1990) have reported that the infection of carrot roots and leaves with the bacterial pathogen *Erwinia carotovora* results in a rapid increase in the mRNA levels of cell wall invertase, with maximal expression observed one hour after the first contact with the pathogen. Similarly, Chou *et al*.

(2000) have reported an induction of the cell wall invertase *Atβfruct1* in *Arabidopsis* leaves infected with white blister rust (*Albugo candida*), while Fotopoulos *et al*. (2003) have shown a similar induction of *Atβfruct1* transcripts in *Arabidopsis* leaves infected with powdery mildew which correlated with an increase in cell wall invertase activity. Finally, Herbers *et al*. (2000) have demonstrated an increase in cell wall invertase expression in tobacco leaves following infection with potato virus Y. Interestingly, Herbers and his colleagues have observed that the induction of cell wall invertase mRNA expression occurred before any increase in enzyme activity (2 and 6 days post infection, respectively), indicating that *de novo* gene expression must make a major contribution to the activity.

The induction of invertase in plants infected with pathogens may be a signal that converts infected organs, tissues or cells into a sink where carbohydrates are needed and, as a result, sucrose is rapidly unloaded (Tymowska-Lalanne & Kreis, 1998a). Infection is known to cause a respiratory rise that is associated with *de novo* synthesis of enzymes (Smedegaard-Petersen, 1984). It is likely that the increased respiratory activity in infected tissues requires a greater utilization of stored or imported carbohydrates; and an increase in invertase may be involved in the mobilization of stored sucrose that makes the rapid rise in respiratory activity possible (Benhamou *et al*., 1991), ultimately providing additional energy required for the activation and establishment of a cascade of defense reactions (Benhamou *et al*., 1991).

CONCLUDING REMARKS

It is clear that invertases have very important roles in plant metabolism, growth and development, and that higher plants contain multiple genes encoding these proteins. The use of different functional approaches has led to an improvement of our knowledge on invertase function. Despite these accomplishments, some crucial questions still need to be answered, such as why there are invertases with different properties localized in different subcellular compartments and how these enzymes interact with each other. Answers to these questions may only arise from multidisciplinary approaches, and most likely will include combined application of physiological, biochemical, and molecular techniques. Invertase knock-out mutants have already been isolated (Sherson *et al*., 2003), and detailed analysis of these mutants should provide important information on the function of individual invertase enzymes. The knowledge gained will help to understand one of the most fundamental processes in plants, which may allow the successful biotechnological manipulation of carbohydrate metabolism and partitioning in order to improve yield and quality in crop plants.

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