Complexities of invertases controlling sucrose accumulation and retention in sugarcane

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Sugarcane is one of the most important cash crops capable of accumulating up to 25% sucrose (w/w) on fresh weight basis. Studies in sugarcane have shown that significant effects are exerted on the sucrose cycle by the enzymes, sucrose phosphate synthase (SPS), sucrose synthase (SS) and invertases, collectively responsible for the synthesis and breakdown of sucrose in the various cellular compartments. Although sucrose is synthesized in the cytosol by SPS or SS, its distribution to various degrees between the apoplast, the cytosol and the vacuole of the storage parenchyma is monitored and controlled by invertases (β-fructofuranosidase). In storage sinks, invertase activity is important for creating a sucrose concentration gradient from the phloem to the sink tissue, and then maintaining the storage sink for promoting phloem unloading. With this perspective, precise manipulation of invertases may be adopted to siphon high accumulation of sucrose in stalks. This review highlights the emerging role of invertases in sugarcane and recent upsurge on transgenic research including RNAi approach and their impact on sucrose content in sugarcane.

Keywords: Invertases, sugarcane, sucrose, transgenics.

SUGARCANE (*Saccharum* spp. hybrids) is one of the most important cash crops, accounting for 78% of sugar produced worldwide[1], as well as an increasingly important source for biofuel(s) production[2]. Sugarcane, being a C_4 crop (family Poaceae), is capable of accumulating up to 25% (w/w) of its fresh weight as sucrose under optimum growth conditions[3]. Historically, increases in sucrose yield have been accomplished through conventional breeding programmes; however, these were attained mainly through improvements in cane yield, not in sucrose content[4]. There are several indications that commercial sugarcane is approaching a yield plateau, probably because its natural genetic potential for sucrose accumulation and biomass production (tonnage) has been fully exploited[5,6]. In recent years, with the advent of genomics, the technologies and genetic resources made it possible to target desired gene(s) in a more strategic and specific manner. It is hoped that genetic manipulation approaches would be more effective in producing new varieties with specific and desired traits. Now the challenge of increasing sucrose yield in sugarcane can be met by careful down/upregulation of the enzymes that are involved in sucrose metabolism.

Sucrose is an important component of yield in sugarcane[7]. The quality of sugarcane juice at harvest is determined by the concentration of sucrose, which should be high, and the concentration of non-sucrose components such as other sugars, should be low[8]. Sucrose begins to accumulate in sugarcane internodes when they start elongating and continues until after elongation ceases[7]. During ripening, sucrose concentrations along the entire stalk increase, and the proportion of the stalk containing significant glucose and fructose concentrations decreases[9]. This pattern implies that sucrose metabolism in the stalk also changes during development. Primary sucrose metabolism is governed by several enzymes such as invertase (E.C.3.2.1.26), sucrose synthase (SS, E.C.2.4.1.13) and sucrose phosphate synthase (SPS, E.C. 2.4.1.14)[10]. Although sucrose can be synthesized only in the cytosol by SPS or SS activity, it is facilitated to various degrees between the apoplast, the cytosol and the vacuole of the storage parenchyma, by invertases[11,12]. Sucrose synthase also catalyses the cleavage of sucrose into hexoses. Sucrose synthase is a glycosyl transferase, converts sucrose in the presence of UDP into UDP-glucose and D-fructose. Sucrose synthase is a cytoplasmic enzyme and in plants two closely related isoforms have been reported. Invertase is a hydrolase and cleaves sucrose into two monosaccharides. Invertases (β-fructofuranosidase) have been suggested to be key regulators for sucrose accumulation in sugarcane stem parenchyma[13–16]. The hydrolytic and/or cleavage activities of the invertases and SS in all these subcellular compartments could, therefore, exert an influence on sucrose metabolism, translocation and storage[17] (Figure 1). A detailed report on invertases, primary structures, functions, and roles in plant development and sucrose partitioning has been provided by Sturm[18].

Invertase as a hydrolysing enzyme: hydrolysis of sucrose before storage

Invertases are known to catalyse the irreversible cleavage of sucrose into the two hexoses, i.e. glucose and fructose, utilizing ATP and forming twice as many hexoses as

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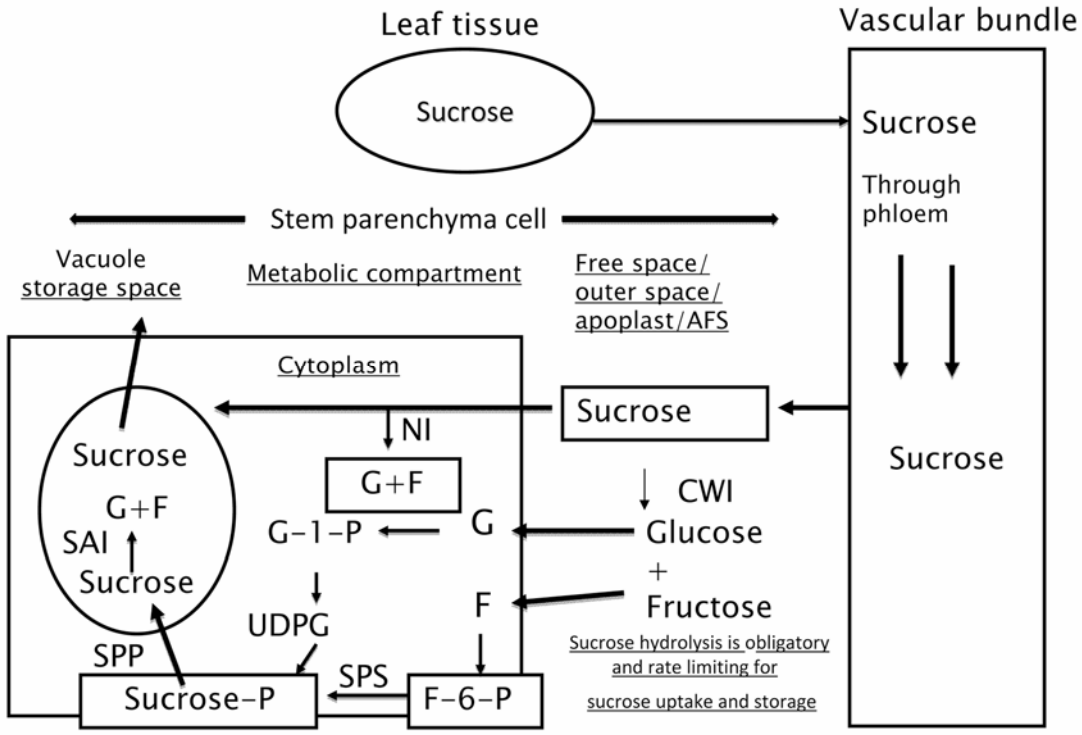

Figure 1. Role of invertases in the synthesis and breakdown of sucrose in sugarcane. SAI, Soluble acid invertase; CWI, Cell wall invertase; NI, Neutral invertase; G, Glucose; F, Fructose; SPP, Sucrose phosphate phosphatase; SPS, Sucrose phosphate synthase; AFS, Apparent free space.

sucrose synthase in the process. The resulting shift in the sucrose : hexose ratio, and consequent signalling from sugar sensors, has been shown to alter the expression of diverse genes[19–21]. Thus, invertases are involved in a wide variety of metabolic processes affecting plant development[22].

In sugarcane, sugar in the form of sucrose is translocated through the phloem to the sinks, where it is used for cell growth, metabolism, respiration or storage. Several different physiological functions have been proposed for invertases, i.e. to provide growing tissues with hexoses as a source of energy[23], to generate a sucrose concentration gradient and to partition sucrose between source and sink tissues, as well as to aid sucrose transport[24]. The invertases hydrolyse sucrose to glucose and fructose, and play a crucial role in the control of metabolic fluxes, sucrose partitioning, and ultimately plant development and crop productivity[18,25–27]. Other possible functions of the invertases include the regulation of cell turgor for cell expansion[28–30] and the control of sugar composition in storage organs[31].

Classification of invertases

Based on their sub-cellular localization, pH optima, solubility and isoelectric point, three different types of invertase isoenzymes could be distinguished: vacuolar soluble acid invertase (VAI), cell wall-bound invertase (CWI), and cytoplasmic neutral invertase (NI)[32–34]. VAI and CWI are of exceptional metabolic importance, as they are the only known enzymes able to cleave sucrose in extracellular compartments such as the vacuole (VAI) and apoplastic space (CWI)[33]. However, NI has previously been considered as a less important 'maintenance' enzyme, involved in sucrose degradation when the activities of VAI and SS are low[35]. Nevertheless, several recent studies in sugarcane have suggested that NI may play a prominent role in sucrose accumulation[36,37]. Furthermore, some of the invertases seem to be involved in the responses of plants to environmental factors, such as wounding and infection[38,39]. Apart from invertases, sucrose synthase also takes part in catabolism of sucrose.

Soluble acid invertase

There are two kinds of acid invertase, both exhibiting optimum activity between pH 5.0 and 5.5, which are located in two separate cellular compartments, i.e. the vacuole (SAI)[5,18] and the apoplast (CWI)[40], also referred to as extracellular invertase. The apoplast enzyme or CWI is confined to the cell wall (ionically bound to the cell wall). It is a key metabolic enzyme involved in the regulation of sucrose partitioning[18,24,41,42]. In sugarcane internodes, CWI probably controls the flow of sucrose from the conducting tissue to the young growing parenchyma cells[13]. CWI is discussed below in separate section. The

primary function of VAI can be characterized in terms of cell turgor regulation and the control of sugar balance in reproductive tissues and mature tubers[21,43]. VAI is also known to play a prominent role in both sucrose import and sugar signalling[21], particularly during the initiation of sink growth and cell wall expansion, when there is greater need for sucrose hydrolysis[34,44]. Several studies in sugarcane have linked VAI activity to the elongation and expansion rates of maturing internodes[45,46].

SAI is concerned with the rate of return of sugar from storage and is believed to be important in the regulation of hexose levels in certain tissues[13,14]. Vacuolar and cell wall invertases show the same biochemical properties, namely pH optima of 4.5–5.0 and cleave fructose residue from disaccharides. These two invertases also hydrolyse other β-fructose-containing disaccharides like raffinose and stachiose. In sugarcane, the SAI enzyme is probably the most extensively studied of the invertases, mainly because it is located in the storage compartment of sucrose. The SAI activity shows marked seasonal variation, being high when growth is rapid and low otherwise[47–49]. The *Saccharum* group that retains high levels of SAI activity in mature stems usually does not store high levels of sucrose[50,51], but sometimes the reverse is true. Apparently, SAI must be low before sucrose can accumulate and this high sucrose accumulation can be the result of other factors as well[15,51]. SAI may play a role in the remobilization of stored sucrose from the vacuole[14] and is believed to be important in the regulation of hexose levels in certain tissues[52]. Mature sucrose-storing internodes of sugarcane contain negligible SAI levels[13], but do, however, exhibit significant sucrose synthetase and cytosolic neutral invertase (NI) isoform with optimum activity at pH 7.0 (ref. 53). The activity of SAI is usually high in tissues that are rapidly growing, such as cell and tissue cultures, root apices and immature stem internodes[13,16,54]. It is also thought to mediate remobilization of sucrose from storage for maintaining cellular processes during periods of stress such as delayed harvest[5]. It has been reported that the inversion of sucrose occurs in harvested sugarcane internodes[55,56]. Earlier studies reported that the sucrose content of mature sugarcane stem internodes was negatively correlated to soluble acid invertase activity, which was considered to be confined primarily in the vacuole. However, in these studies, the sugarcane plants used for comparison were either different genotypes, or grown under different environments, or sampled at different developmental stages, all of which are known to affect the level of SAI activity. Therefore, conclusions drawn from these studies were not certain.

Unlike sugarcane, in sugar beet sucrose is not hydrolysed by invertase prior to its uptake from the apoplast. The uptake into the cytoplasm of this tissue is passive. Although acid invertase is present in young sugar beet, this enzyme is absent in sucrose-storing beet; whatever sucrose is cleaved there, it is by sucrose–UDP glucosyl transferase, i.e. sucrose synthase reversal. Since in beetroot the activity of sucrose phosphate synthase is very low, in all probability this enzyme is not involved in sucrose transport into the vacuole for storage. Based on high vacuolar concentration of sucrose in sugar beet, it is suggested that during storage the transport of sugar into the vacuole is by active transport.

Previously, Hongmei *et al.*[57] have reported that increased invertase activities in the cell wall and in the cytosol cause a decrease in the sucrose content in the sugarcane callus cells. Further, they have tried to suppress the SAI activity by introducing an anti-SAI construct into sugarcane cells and then determined the resulting sucrose level. A sugarcane-soluble acid invertase cDNA, scinvm, was isolated from Molokai 5829 (*Saccharum robustum*), a low sucrose accumulating variety. A portion of the cDNA was placed under control of the maize Ubi-1 promoter in the antisense orientation. This construct was transformed into sugarcane embryogenic calluses derived from H62-4671, a high sucrose accumulating cultivar. The antisense gene suppressed acid invertase activity up to 50% in the soluble fraction and 25–30% in the cell-wall fraction in the cultured cells. In transgenic cells, the sucrose level was increased about two fold. These results indicated that SAI activity is indeed a limiting factor in the sucrose accumulation process of sugarcane.

Cell wall-bound invertase

The extracellular cell wall-bound isoforms of acid invertase are associated with rapidly growing tissues and are thought to participate in the apoplasmic–symplasmic translocation of sucrose[14,15]. A strong correlation between the rate of cell extension and CWI activity has been reported, as this enzyme is the gateway for the entry of sucrose into the cell in juvenile tissues[15]. It plays a key role in phloem unloading and assimilate uptake, specifically in sink tissues where an apoplastic step is involved[22,58]. As CWI cleaves apoplastic sucrose, it ensures a steep concentration gradient of sucrose from source to sink[24], and has been consequently implicated as a pivotal component in establishing or creating such metabolic sinks[58]. Furthermore, the enzyme has been implicated in maintaining a strong source–sink relationship[58]. The role of CWI has been shown to be highly prominent in developing seeds and pollen, where a gap in plasmodesmatal connections between cells has been shown to exist and apoplasmic sugar transfer is prominent[20]. However, if at least some sucrose moves across the cell-wall space, CWI can additionally influence sinks where the plasmodesmatal connections remain intact, such as in developing carrot root and potato tubers[21,59]. The importance of CWI in sugar partitioning has also been examined in maize mutant (*miniature1*) which, due to an abolishment of

endosperm-specific CWI, showed a small seed phenotype[60]. Similarly, antisense inhibition of CWI in carrot checked tap-root formation[27]. In both cases, apoplasmic hexoses were required for normal development. A variety of isoforms of the CWI family have been isolated[58]. Similar to SS, the CWI isoforms exhibit highly tissue-specific mRNA expression patterns. In maize, four isoforms that differ greatly in expression levels have been identified (*Incw1*, *Incw2*, *Incw3* and *Incw4*)[61]. Expression of *Incw3* and *Incw4* is very low in grain, as it is detectable only by reverse transcription–polymerase chain reaction (RT–PCR)[62]. In tomato, the expression of CWI isoform *Lin7* is observed solely in anther tapetum cells and pollen grains[63]. Anther-specific isozymes of CWI are furthermore evident in both tobacco and potato[64,65], indicating the crucial function for extracellular invertases in providing carbohydrates to the male gametophyte. Recently, antisense reductions of CWI expression in pollen have demonstrated the important role of CWI in maintaining male fertility[58].

In sugarcane, CWI has been studied in detail by Lingle[66]. Results obtained indicated increased CWI activity, sucrose and sucrose to total sugar ratio with an increase in internode age. Similar results have been also reported by Botha *et al.*[67]. However, Gayler and Glasziou[15] proposed a cleavage and resynthesis model for sucrose unloading and storage in sugarcane. In this model, sucrose is unloaded into the apoplast of the storage parenchyma, where it is cleaved into glucose and fructose. The hexoses are then transported into the cell, where sucrose is resynthesized. Lingle[68] demonstrated that sucrose could be transported intact in sugarcane, but did not show that cleavage and resynthesis played no role in sucrose storage. It remains possible that cell-wall acid invertase contributes to sucrose storage in sugarcane. Higher activity of cell-wall acid invertase in high sugar genotypes enhances sucrose unloading into the internode tissue.

Expression of genes involved in sucrose metabolism relative to 18s rRNA was compared in developing internodes of a high sucrose genotype, Muntok Java (MJ), and a low sucrose genotype, PIN84-1 (PIN), using quantitative PCR. In general, expression of all genes of the sucrose cleavage enzymes, sucrose synthase (Sus1 and Sus2), soluble acid invertase (Ivr1 and Ivr2), and cell-wall acid invertase (Cwin1), as well as genes for sucrose phosphate synthase (SPS1 and SPS2) which synthesizes sucrose were greater in high sugar genotype MJ than in PIN. The sucrose transporter gene, *Sut1*, had relatively low expression in the very young internode of both the genotypes, and its expression increased with internode age, especially in the higher sucrose genotype. The internodes of the high sucrose-storing genotype appear to be metabolically more active than those of the low-sucrose genotype[66]. Thus, the cell-wall invertase gene may be a good candidate for improving sucrose accumulation in sugarcane.

Neutral invertase

The soluble neutral invertases (NI) are involved in cytosolic sucrose hydrolysis. Contrary to other invertases, studies on NI have been largely neglected[11]. However, the enzyme has been purified and characterized in a variety of species[69–72]. The cycling of carbon between sucrose and hexoses, as a result of simultaneous synthesis and degradation of sucrose has been demonstrated in the cytosol of sink tissue in several species, including sugarcane[73–75]. This 'futile cycling' of sucrose is believed to be primarily responsible for overall sucrose accumulation in sugarcane, which may be mediated by SS and/or NI[76]. A more recent correlation between hexose levels and NI in younger tissue has suggested that the enzyme could contribute to the supply of hexoses in younger culm tissue, thus implicating the enzyme as an important component of growth and metabolism[56,72]. The cDNAs encoding an enzyme with neutral/alkaline invertase activity have been cloned in poison rye grass (*Lolium temulentum* Lam. Kuntze) and carrot[18,77]. However, further cloning from other species will be required to elicit whether the neutral/alkaline invertases cover more than one family of enzymes. Transcripts of NI have been found in all sugarcane tissues, although at relatively low levels[36]. The highest levels of NI expression and activity were observed in maturing culm, where sucrose was low and hexoses were high, with decreasing activity as the culm matures[72]. A similar pattern of distribution was seen in carrot, where the highest steady-state levels of NI favoured young, actively growing tissues[18]. The relationship between NI and its substrates throughout the sugarcane growth season is still not clear[72,76]. This has led to the suggestion that NI activity may only be linked to local environmental and cyclic factors, rather than development and maturation[72].

According to Vorster and Botha[71], NI activity in sugarcane was detected in the 60, 120 and 240 kDa forms. NI is located in the cytosolic compartment, where it functions optimally at pH 7.0. Early studies found that NI activity (expressed on a fresh weight basis) increases with internode maturity and, therefore, correlates positively with sucrose concentration in internodal tissues[15,52,76,78]. However, conflicting data have also been reported where NI activity (also on a fresh weight basis) decreases with internode maturation[79]. The most recent studies report that NI activity (on a per mg protein basis) increases up to the fifth internode before declining as the internodes mature further[71,80]. In addition, NI is reported to be the only sucrolytic enzyme that shows positive correlation with hexose levels[15,36] and negative correlation with sucrose[72]. As NI is a cytosolic enzyme, it is the only invertase that functions in the compartment where sucrose is actively synthesized and could, therefore, play a crucial role in the process of sucrose accumulation in sugarcane. In contrast to vacuolar and extracellular inver-

tases, neutral invertase is not a β-fructofuranosidase and appears to be sucrose-specific[18]. Moreover, a kinetic model for sucrose metabolism in sugarcane identified NI as the key enzyme in determining the flux through this cycle[81]. Although only theoretical and speculative in nature, this *in silico* model suggests that NI is a suitable target for a reverse genetic approach aimed at increasing sucrose content in sugarcane internodes. Conflicting reports of the distribution of sugarcane neutral invertase (SNI) activity in sugarcane indicated that in the culm tissues where sucrose content was low and hexoses were high, NI transcript and protein levels were higher than sucrose storage tissues[36]. The discovery of significant levels of NI activity in mature sugarcane stem tissues has led to the hypothesis that NI may affect control over the expression of sugar-responsive genes in mature internodes by controlling the hexose concentration of the cytosol[71]. Recently, the establishment and characterization of transgenic sugarcane suspension cultures with reduced NI activity has been described[82,83]. These suspension cultures showed reduced sucrose cycling, impaired growth characteristic, increased sucrose concentration and reduced hexose levels, indicating a decline in partitioning of carbon to the respiratory pathways. Existing studies on plants where invertase activity has been genetically modulated in the cytosol of a higher plant described the effects of over-expressing a yeast invertase in the cytosolic compartment of transgenic potato and tobacco plants[25,57,84]. In these cases, the upregulation of cytosolic invertase activity resulted in an increase of respiration rate. Rossouw *et al.*[83] presented evidence that NI in sugarcane possessed crucial importance for providing carbon backbone for respiration and sucrose cycling.

Regulation of expression of invertases

Studies have indicated that the steady-state level of invertase activity is regulated by several complex and unique regulatory mechanisms[85]. Thus, the temporal and spatial regulation of invertases has its own significance.

Vacuolar acid invertase

The expression of VAI is dependent on the multitude of signals, including sugars (particularly hexoses), hormones and other environmental stimuli[19,22,48]. The repression of VAI expression during drought has also been linked to carbon resource management during reproduction[86]. As sucrose cleavage in the early phase of maize, kernel growth has been shown to be predominantly controlled by VAI before the upregulation of other sucrose metabolism genes the onset of water stress often causes younger kernels to abort, thus giving preferentiality to the survival and development of more mature kernels[86]. A variety of gene isoforms exist for VAI, which have been shown to have different developmental and tissue-specific expression patterns. The precise function of each isoform remains to be fully elucidated. In species other than maize, a considerable variation of VAI activity has been observed, particularly in the photosynthesis of fully expanded leaves[87]. Furthermore, Huber[87] has demonstrated that only species with a low VAI activity accumulate sucrose in leaves, as an end-product of photosynthesis. As sugarcane favours accumulation of photoassimilate in the form of sucrose, a limited activity for VAI in sugarcane leaves is expected. Several challenging advances have been made in identifying novel regulators of VAI at the protein level, including the discovery of a pre-vacuolar regulatory system in *Arabidopsis*[88]. Prior to delivery in the vacuole, VAI may be compartmentalized for extended periods in a vacuole-associated endomembrane vesicle known as the precursor protease vesicle (PPV). The PPV is also home to an inactive form of a vacuolar processing enzyme (VPEy protease), which may be released into the vacuole together with VAI. VPEy protease auto-activates upon entering the vacuole and can then target VAI for degradation. Thus, the PPV compartment not only plays a role in regulating the time at which VAI activity commences, but also controls its vulnerability to subsequent turnover by VPEy protease[88].

Cell wall invertase

Several regulatory mechanisms have been revealed for CWI, including tissue-specific expression[89], differential transcript formation[89], exon skipping[90] and stimulation of activity by a variety of phytohormones[58]. Importantly, CWI expression and enzyme activity are also modulated by sugars. Roitsch *et al.*[91] observed higher enzyme activity and increased levels of CWI mRNA in the presence of sucrose and glucose using photoautotrophic suspension cultures of *Chenopodium rubrum* (L.), whereas various isoforms of CWI have been upregulated by glucose in tobacco and *Arabidopsis*[34,92] and by sucrose in tomato[63]. More recently, research in tomato suspension cultures has observed upregulation of a CWI isoform using non-metabolizable sucrose analogues, such as palatinose, turanose and flourosucrose[93]. However, both turanose and palatinose are synthesized by plant pathogens, indicating that the invertase response may be linked to stress-related stimuli, rather than a unique sugar signalling mechanism. Nevertheless, as both metabolizable sugars and stress-related carbohydrate stimuli have been shown to regulate CWI expression, CWI is potentially an important marker gene for the analysis of converging signalling pathways[58]. CWI activity is regulated through a protein–protein binding complex between CWI and invertase inhibitor proteins (INH)[43,92]. Expression analysis of CWI and INH has indicated that, at certain stages of plant development,

CWI activity is downregulated by INH, the latter operating as a regulatory switch[92]. INH was originally isolated and purified to homogeneity from tobacco leaves, whereas the genes encoding INH have been cloned from *Arabidopsis* and tobacco[43]. Recently, the isolation of INH-type isoforms in several plant species has provided evidence that independent INH-systems regulate both CWI and VAI activities[33,94]. Although the exact physiological mechanism of INH is still unclear, one possibility is that it may function to modulate CWI activity under adverse conditions, such as maintenance of a minimal, critical sucrose concentration during sugar starvation[35].

Neutral invertase

NI is regulated by feedback inhibition by the products of the hydrolytic reaction, namely glucose and fructose[70]. Product inhibition of NI would only be significant at the cytosolic hexose concentrations of internode 2 to 10, and would have virtually no impact at the symplastic hexose concentrations of internode-20 and greater[12].

According to Resende *et al.*[95], trinexapac-ethyl (TE), one of the ripeners reduces endogenous levels of an active form of gibberellic acid in sugarcane. Gibberellic acids play an important regulatory role in the activities of invertases in different plant species[34]. Therefore, the major physiological response of sugarcane to TE treatment is stem elongation inhibition and possible changes in the photoassimilate partitioning, favouring sucrose accumulation[96]. The activity of CWI systematically decreased after TE application. Hormones such as auxin or cytokinin have increased the acid invertase activity in several plant species[18].

Metal ions affecting invertase expression

Among the different metal ions tested, manganese chloride strongly inhibited the activity of all soluble acid invertase isoforms, and thus may be useful to induce early maturity and controlling sucrose inversion in sugarcane, thereby increasing sugar recovery[97,98]. Vorster and Botha[71] reported inhibitory effects of Hg^{2+} on the activity of sugarcane neutral invertase. Application of 0.005 M $FeCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$ and $AlCl_3$ reduced invertase activity by 80%, 73%, 32%, 45% and 22% respectively, in sugarcane stalk[99]. Foliar application of Mg^{2+} and Mn^{2+} ions reduced SAI expression and increased sucrose per cent juice, S/R ratio and CCS % juice in sugarcane stalk of a low sugar genotype BO 91 (ref. 100).

Expression of invertases after cane harvest: post-harvest deterioration

Post-harvest sucrose losses have been reported from many cane-producing countries and linked with low sugar recovery and problems during sugar processing. Biodeterioration is associated with the inordinate delays between harvest to milling of sugarcane, aggravated by many intrinsic and extrinsic factors causing enormous depreciation in cane tonnage as well as sugar recovery. Besides harvest-to-mill delays, other factors such as ambient temperature, humidity, cane variety, period of storage, activities of invertases, maturity status, etc. are responsible for decline in sugar recovery. The activity of invertases and proliferation of acid, ethanol and polysaccharides (dextran) producing microbes play a crucial role in the loss of recoverable sugars in stale cane and milled juice[101].

During late crushing operations (after March) when the ambient temperature is high (>40°C), SAI showed significant rise in stand-over crop and harvested cane[102–105]. Early experiments have demonstrated the presence of acid and neutral invertase in cane stalk and both the enzymes have the tendency to increase after harvest[102,103]. Batta and Singh[105] reported seven fold increase in activity of acid invertase compared to four fold increase in neutral invertase after 12 days of storage. Solomon *et al.*[104] noticed increase in the activity of both acid and neutral invertase after 72 h of storage of cane, with a corresponding rise in the level of invert sugar. Eggleston and Legendre[106] advocated that the enhanced activity of acid invertase could be due to possible synthesis of cut-induced invertase and decreased activities of sucrose-synthesizing enzymes induced by pH change. Enhancement in the acid invertase activity during storage of harvested cane was higher in all the genotypes under ambient environment from 24 to 96 h staling compared to the freshly harvested crop. It has also been noted that the acid invertase activity enhanced dextran formation[101,106]; therefore, downregulating this enzyme could minimize sucrose losses after harvest.

Efforts have been made to reduce loss in tonnage and sucrose inversion using physico-chemical methods. These include spraying of water, bactericidal solution, use of anti-inversion and anti-bacterial formulations and pre-harvest foliar and soil application of zinc and manganese compounds. An integrated mill sanitation programme and simultaneous use of dextranase could further improve sugar recovery and minimize problems caused by dextran. The possibility of electrolysed water (EW) fogging to reduce post-harvest deterioration in field and mill yard has also been explored[107]. Some of these methods are useful and present larger options for the industry to minimize after-harvest quality losses in the field and milling tandem[101].

Transgenic research

Strategies to increase sucrose concentration in sugarcane via transgenic manipulation require a broader understanding of the processes involved in sucrose accumulation,

including the possibility that culm sucrose accumulation may be regulated by sink (including storage) demand[108]. Most efforts to manipulate sugarcane sucrose concentration by transgenesis have targeted single genes encoding putative rate-limiting sucrolytic enzymes in the culm. These include soluble acid invertase[109], neutral invertase[82,83], pyrophosphate-dependent phosphofructokinase[110], and a yeast invertase carrying various leader sequences for targeting to the apoplast, cytosol or vacuole compartments[57]. Despite successful transformation, transgenic plants did not result in a significant increase in overall culm sucrose accumulation[109,110]. It is suggested that attempts to increase sucrose content of sugarcane by the transgenic manipulation of sucrose metabolism enzymes, whether singly or in tandem, must take cognizance of regulatory feedback mechanisms known to exist in other plants[111–114]. This has, of late, been proposed by McCormick *et al.*[115,116] in sugarcane. To date, attempts to increase sucrose content in the sugarcane culm through the modification of carbon flux and partitioning have ignored this potential regulatory feedback between the culm (sink) and the leaf (source)[117].

Papini-Terzi *et al.*[118] have reported many sugarcane genes associated with sucrose content and also indicated overlap of these genes with drought and cell-wall metabolism processes. To classify the role of these genes as well as to define targets useful for sugarcane improvement, transgenic research is needed. By utilizing sucrose isomerase and proline synthase genes in sugarcane, transgenics for increased sugar concentration and water stress tolerance respectively, have been reported[119,120]. Under these cases increased sugar concentration due to additional accumulation of isomaltulose, a high-value sugar vis-à-vis increased photosynthesis, sucrose transport and sink strength has been observed. Similarly, higher biomass yields after 12 days of withholding water was observed along with tolerance to water stress.

Controlling the level of invertases at suitable locations is the need of hour, which can be initiated utilizing the RNAi approach. The reduction of invertase activity soon after harvest of sugarcane crop could be useful in minimizing the post-harvest sucrose losses, and it can be initiated by implicating the RNAi approach. The inversion of sucrose into glucose and fructose is the major problem in stale cane, which led to significant loss of sucrose. Another problem which comes across after crushing the stale cane is the production of dextran. To minimize this, dextranase is recommended; however, generating transgenic plant using appropriate dextranase gene with appropriate promoter will restrict the production of dextran.

Future prospects

Future research at improving sugarcane sucrose accumulation should examine the role of these sugar metabolizing systems for potential transgenic manipulation of sugarcane sucrose accumulation. From an agricultural perspective, the potential definitely exists to increase the yield of sucrose, although careful consideration should be made of the growth penalty incurred in the transgenic plants. Future prospects would include the use of inducible or tissue-specific promoters in genetic transformation for a more controlled manipulation of invertases in specific tissues. There is a need of looking at all three invertases (SAI, CWI and NI) in the context of source–sink communication, as it is now amply clear that the strength of the sink controls the source activity. Hence it is more important that plant physiologist should look into the perspective of combing the research carried out by molecular biologists or plant biochemists while addressing the changes happening at the physiological as well as enzymatic (gene) level during the growth and accumulation of sucrose in mature cane. The differential accumulation of sucrose in the stalk of sugarcane cultivars and related *Saccharum* species, namely *S. officianrum* and *S. spontaneum* (the former has high sucrose in stalk, but exhibits low rate of photosynthesis compared to that of the latter) would provide base genetic materials to reveal the complexities of invertases and their impact on overall sucrose accumulation in sugarcane. Transgenic research will finally validate and generate products of such research.

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