# Time-course expression of soluble acid invertase (SAI) gene mirroring post-harvest cane quality deterioration: effective treatments cause reduction of SAI gene expression

Sugarcane is a crop valued mainly for the high sucrose content in its cane stalks. Since sucrose is the most essential part of commercial cane sugar (CCS), it automatically becomes the top priority for farmers, and consequently, for breeders and agronomists as well. In the mature internodes, sucrose content can reach around 25% of the fresh weight of the culm. However, the rapid inversion and deterioration of sucrose in harvested cane, makes it a perishable commodity. This deterioration is associated with a variety of factors such as ambient temperature, humidity, cane variety, period of storage, activities of invertases, maturity status, etc.[1]. However, the sucrose loss mainly occurs due to endogenous invertase as well as invertase of bacterial origin (resulting from bacterial invasion through the cut ends or damaged sites of stalk) and consequent production of secondary metabolites such as organic acid, dextran, gum and alcohol. This biochemical process becomes pronounced with the passage of time, making the cut-to-crush time lag of crucial importance for maximum sugar recovery. The problem has been alarming, especially in India, as the delay between harvesting and milling of cane sometimes even exceeds the usual 3 days, incurring huge loss due to cane staling[2] (Figure 1). Studies have reported up to 2.0 units decline in CCS% in cane, within a period of 72 h after harvest, depending upon the sugarcane genotype and milling season[3]. This ultimately leads to a loss of nearly Rs 30 lakhs every day, to a mill having crushing capacity of 5000 tonnes of cane per day (TCD).

In the recent past, various physical/chemical methods have been tried to cut down the post-harvest sucrose losses, but their practical use has mostly been restricted by availability, high cost and environmental problems. In the past, many authors have discussed the effect of trash cover, water spray and shade effects in minimizing the sucrose losses in sugarcane[4,5]. Spraying of water is perhaps considered beneficial because invertase activity is said to go up, with the loss of moisture. Electrolysed water (EW) prepared by electrolysing saline to create a disinfectant, has been reported to cause lesser reduction in post-harvest storage quality of sugarcane and lesser formation of invert sugars compared to untreated cane[1,6]. Various bactericides such as formaldehyde, DBAC, IFOPOL, DNDT, potassium permanganate and sodium metasilicate, Tsunami-100, Sucroguard, etc. have been suggested to curb deterioration of cane and milled juice. Solomon *et al.*[7] have reported the efficacy of a few chemical formulations containing both antibacterial (quaternary ammonium compounds/thiocarbamates) and anti-inversion chemicals (sodium metasilicate/sodium lauryl sulphate), thus equipped to check post-harvest sucrose losses (both microbiological and biochemical). These aqueous formulation(s) are sprayed over freshly harvested cane (whole stalk and billets) and the treated cane is covered with a thick layer of dried cane leaves (trash). Formulation containing benzalkonium chloride (BKC) + sodium meta silicate (SMS) has been found to be most effective – the synergistic effect of antibacterial and anti-inversion chemicals reduces the sucrose loss from harvested cane, up to a period of one week, irrespective of storage temperature and variety[7–9], improving sugar recovery by about 0.5 units[1].

Solomon *et al.*[10] observed increase in the activity of both acid and alkaline invertase after 72 h of post-harvest storage of cane, with a corresponding rise in the level of invert sugars. Studies have reported increase in invertase activity in post-harvest storage of cane[11–13]. In sugarcane, soluble acid invertase (SAI) plays a major role in controlling sucrose content in cane stalk vacuoles through sucrose import and sugar signalling, particularly during the initiation of sink growth and cell-wall expansion, when

**Figure 1.** Massive sucrose loss due to delayed cut to crush of sugarcane.

there is a high need for sucrose hydrolysis, as well as during maturation of the plant. Thus, SAI activity is high in storage tissues that are rapidly growing during internode growth and development, and low otherwise[14]. Hence, mature, sucrose-storing internodes of sugarcane contain negligible SAI levels. However, SAI is also thought to mediate remobilization of sucrose from storage, for maintaining cellular processes during periods of stress, such as delayed harvest.

Thus, studying the expression and regulation of SAI, especially during the post-harvest (sucrose inversion) period, can be of importance in minimizing the invertase-ridden sucrose loss. The changes in the transcript levels of SAI may be precisely estimated by employing quantitative RT-PCR tool (using gene specific primers), besides other intrinsic biochemical factors. In a preliminary effort in this direction, Chandra *et al.*[15] analysed the DNA sequences of soluble acid invertases of 13 crops species for sequence homology and based on the most conserved gene region, designed six primer pairs (forward and reverse). Utilizing one (SAIF1/R1) of these six primer pairs, they generated the first ever SAI gene sequence, specifically for *Saccharum spontaneum* SES34 (accession no. KC570328) and also for *Saccharum* spp. hybrid CoJ64 (an early maturing and high sucrose accumulating variety of sugarcane) and *Saccharum officinarum* 28NG210 (accession nos. KC570326 and KC570327 respectively)[16].

Based on earlier findings a study was conducted at the Indian Institute of Sugarcane Research, Lucknow, in early March 2013, mirroring the harvest-to-mill delay and drawing a distinct comparison, at the molecular level, of the effect of various physical/chemical treatments on the expression of SAI, in particular. The study aimed at using expression/suppression of SAI, as a measure to assess the effectiveness of different physical/chemical treatments, in controlling inversion of sugar due to cane staling over a period of time. Canes of uniform size, of the early maturing variety CoLk94184, were harvested, topped and piled in four separate bundles of 10 each and a freshly cut, untreated cane (0th day) was used as such. Each of the bundles was subjected to a different physical/chemical treatment, viz. T-1: cane sprayed with water and kept in the open (control), T-2: water-sprayed cane covered with trash, T-3: cane sprayed with electrolysed water, T-4: cane sprayed with chemical (0.1–0.2% BKC + 0.2–0.5% SMS). A cane was drawn from each bundle and a small amount of tissue from the 5th internode (from the bottom) of each (because massive bacterial infection is found up to 6 inch from the cut ends, after about 1½ h of storage) was used as sample for RNA extraction. Juice extracted from the remaining portion of each of these (5th) internodes was used to estimate reducing sugars by the spectrophotometric method of Nelson[17]. Also, commercial cane sugar was calculated using the formula[18]: 1.022 (pol% juice) – 0.292 (brix). This experiment was done with the harvest (0th) day cane and repeated at intervals of 72,144, 168 and 192 h, after harvest.

RNA was extracted using TRIZOL reagent (Invitrogen, USA), according to the manufacturer's instructions. The quality of RNA samples was verified on 1% agarose gel and RNA quantification was done using the Q5000 spectrophotometer (Quawell Technology, USA). A qRT-PCR of these RNA samples was run using the Qiagen one-step RT-PCR kit, employing the SAI gene-based primer F: 5′-ATGGCCCGGTGTACTACAAG-3′ R: 5′-AGCGCGTAGTAGTCATGTCG-3′ (from *Saccharum* spp., accession no. AY302083) to determine how SAI, in particular, takes part in sucrose inversion in response to the various physical/chemical treatments. Interpreting the gel electrophoresis results (Figure 2), the freshly cut, untreated cane showed no SAI expression, indicating that its activity

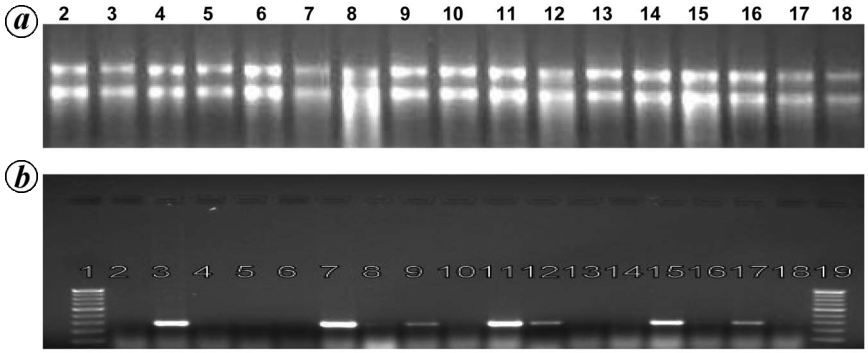


**Figure 2.** ***a***, Agarose gel electrophoresis of total RNA (lanes 2–18) isolated from fresh and stale canes (both treated and untreated) depicting quality and quantity of RNA used for qRT-PCR analysis. ***b***, qRT-PCR analysis using total RNA and SAI gene-based primer pair. Lanes 1 and 19, 100 bp DNA ladder as molecular weight marker; lane 2, Freshly cut and untreated cane; lanes 3, 7, 11, 15, Cane sprayed with water and kept in open (control) for 72, 144, 168 and 192 h of post-harvest storage respectively; lanes 4, 8, 12, 16, Water-sprayed cane covered with trash and left for 72, 144, 168 and 192 h of post-harvest storage respectively; lanes 5, 9, 13, 17, Cane sprayed with electrolysed water and left for 72, 144, 168 and 192 h of post-harvest storage respectively; lanes 6, 10, 14, 18, Cane sprayed with chemical (BKC + SMS) and left for 72, 144, 168 and 192 h of post-harvest storage respectively.

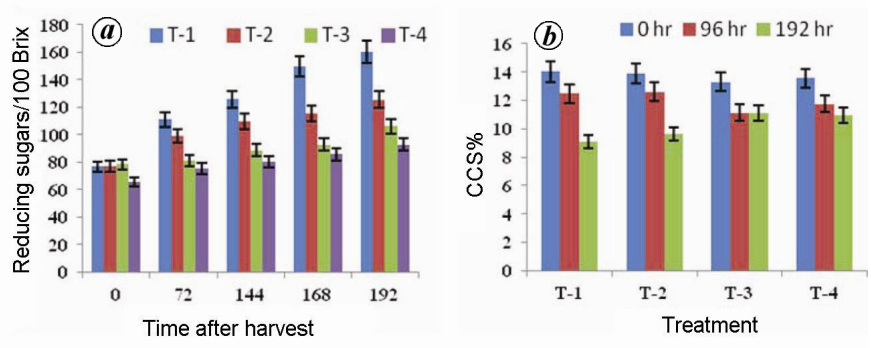


**Figure 3.** Reducing sugar (***a***) and CCS%, (***b***) estimated in fresh and stale canes, depicting decline in cane quality with increasing duration of post-harvest storage. T-1: cane sprayed with water and kept in the open (control), T-2: water-sprayed cane covered with trash, T-3: cane sprayed with electrolysed water and T-4: cane sprayed with chemical (BKC + SMS).

goes up only on time lag. The water-sprayed, open cane (control) showed sharp bands in all samplings, pointing to poor protection from SAI inversion. On the other hand, the water-sprayed, covered cane and those sprayed with electrolysed water were protected in the initial phase of the study, but were found to show comparatively weaker SAI expression recorded 144 h after harvest. However, an appreciable suppression of SAI expression was observed over the entire study period of 192 h (=8 days), especially in response to the BKC + SMS combination treatment, evident from the fact that no bands were observed with respect to SAI in any of the lanes corresponding to the T-4 treatment.

Reducing sugars in juice are considered an important indicator of cane deterioration. Solomon *et al.*[8,9,19] have also reported higher levels of reducing sugars in juice on storage of harvested cane. Also, CCS is the major quality determining factor which is considered while studying the deterioration. Thus, alongside the expression analysis, reducing sugars and CCS% were also estimated in juice samples to ascertain the deterioration in cane quality. As shown in Figure 3, at the time of harvest, reducing sugars were recorded as 76.65/100 Brix which, over the period of 192 h, increased by 2.09 fold in control cane (T-1). However, in T-2, T-3 and T-4 canes it increased by 1.63, 1.42 and 1.35 fold respectively. CCS% at the time of harvest was calculated as 14.01 which decreased by 4.91, 4.29, 2.59 and 2.23 in T-1, T-2, T-3 and T-4 canes respectively, in the span of 192 h post-harvest.

These results reassert the efficacy of chemical treatments with a synergistic anti-bacterial and anti-inversion effect, in minimizing post-harvest sucrose loss, especially biochemical inversion. However, since the inversion of sucrose into glucose and fructose is the major cause of significant loss of sucrose in stale cane, future research must be targeted towards increasing sucrose yield by careful down-regulation of the enzymes, especially SAI, involved in sucrose inversion. With the recent advancements in genomics anti-sense technology developed to reduce invertase activity soon after harvest of the cane crop, would be useful in minimizing the post-harvest sucrose losses. Such control can be realized by employing the RNAi approach to tune the level of SAI at suitable locations, soon after harvest, which will have far-reaching impact on the sugar industry vis-à-vis export policy of the Government.


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# Transformation of colourful pattern of eyespot in peacock wing

An eyespot or ocellus is an eye-like pattern or structure found in various phyla, including butterflies, reptiles, felids, fishes and birds[1–3]. In some species of fishes and butterflies, eyespot is a form of mimicry to draw a predator's attention away from the most vulnerable body parts, or to appear as an inedible or even dangerous animal[2,4–6]. In some butterflies, besides antipredatoty function it also plays an important role in kin recognition and sexual selection[5,6]. In birds like the peacock, eyespot is present in the tail feather with a function of intraspecific communication and courtship. The communication is mediated by the fan of the tail feather, which is composed of 170 eye feathers bordered by the 30 T-shaped feathers which do not contain an eyespot[7]. All the eye feathers are arranged in an increasing length so that all the eyespots will be visible when the tail feather is fanned[7]. The eyespot of the peacock is different from other animals as it includes an intricate shape and multiple rings having bright and iridescent colours (colours that change with the viewing angle). The peacock has various feather patterns throughout its body. Feather patterns observed in birds are diverse in