explanation to other age-old medicinal practices too? It is up to the National Center for Complementary and Alternative Medicine to answer such an appropriate query. The scientific world is of the opinion that further research has to be carried out to replicate the work done at IIT-Bombay. Only then, the proposed hypothesis of Bellare and his group based on nanotechnology can attain the status of an accepted theory.

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Revisiting the source–sink paradigm in sugarcane

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Extensive research has been directed towards the basic understanding of accumulation of photoassimilates in defined and undefined storage organs in plants. Biophysiological study pertaining to carbon source (leaf)–sink (specialized storage organs, culm parenchyma tissue in sugarcane) communication and its relationship has been better addressed in C_3 over C_4 crops. Nevertheless, sugarcane (*Saccharum* spp. hybrids), a C_4 crop is the best reference crop where the source–sink relationship study has been largely concentrated owing to its ability to accumulate a highly economical product, i.e. sucrose in culm. At the global level, > 70% sucrose comes from sugarcane[1]. Several authors have opined that accumulation of sucrose in sugarcane is principally regulated at the level of sink (cane stalk/culm). Furthermore, futile cycle of degradation–synthesis of sucrose occurring in the culm is also one factor which controls and regulates sucrose concentration; hence all these cellular activities account for variable accumulation of sucrose in the cane stalk. A better understanding of sucrose synthesis and accumulation in sugarcane and its modulation through exogenous or endogenous means leading to higher sucrose productivity would be a boon for sugarcane farmers, millers and associated industries.

The regulatory enzymes, viz. the three invertases, soluble acid (SAI), cell wall-bound (CWAI), neutral (NI); sucrose synthase (SS) and sucrose phosphate synthase (SPS) play an important role in sucrose metabolism. The hydrolysis of sucrose in tissue-free space (apoplast/apparent free space) seems obligatory and rate limiting for sucrose uptake and its storage, which play a central role in spatial and temporal regulation of sucrose accumulation in sugarcane[2]. Continued research on sugarcane has indicated that attributes like delayed leaf senescence, increased sucrose loading rates in source tissues, high photosynthetic activity (electron transport rate) and higher activity of CWAI are found to be associated with the high total sugar phenotype of a sugarcane line having the ability to accumulate a high level of sucrose in the culm[3]. The maximum level of accumulation of partitioned carbon into sucrose is 0.7 M in the sugarcane culm. Normally, the observed sucrose content on dry matter (SCd) basis is 350–400 mg/g; however, the capacity of some lines to accumulate 500–560 mg/g sucrose (SCd) has also been reported[4]. Physio-biochemical processes like rate of photosynthesis, partitioning of carbon pools other than culm storage (respiratory demand of carbon and demand for water-insoluble compounds), loading and unloading of sucrose in the leaf and culm, three-phasic metabolic activity of sucrose in parenchymatous cells (apparent free space, metabolic space and vacuolar storage space), and developmental constraints such as duration and timing of maturation (temperature, drought-dependent maturation and use of ripeners) may accentuate the ceiling of apparent sucrose concentration in the culm. Thus, the physiological threshold of sucrose in the culm may be seen in the context of feedback regulation by biosensors like hexoses, as well as energetic limitations imposed by continuous cleavage and synthesis within the storage pool[5].

In many countries, including India, improvements in sugarcane have been primarily in cane yield rather than sucrose content, which is one of the major constraints in improving sucrose productivity. In view of the limited area, growing domestic demand for white sugar and necessity of extra ethanol production for blending, it is imperative to augment sucrose productivity per se. There is need for targeted breeding with the objective of increasing the concentration of sucrose in the culm. In this regard Lakshmanan *et al.*[6] and Snyman *et al.*[7] stressed upon extensive R&D in the development and utilization of new molecular techniques for better understanding of the fate of sucrose in the culm and the cellular mechanism that regulates sucrose accumulation. Extensive research in this direction has yielded deposition of more than 3 lakh ESTs sequences from more than 40 libraries in public domain (SUCEST project from Brazil), 1515 genomic[8,9] and 342 EST-based SSR

markers[10]. Several sugarcane associated genes showed overlapping expression with drought[11] and putative metabolic control points for increased culm sucrose accumulation. Although reasonable information is currently being utilized in conventional breeding programmes to improve crop performance[7], no significant progress could be realized in improving the culm sucrose. Grof and Campbell[12] have argued that through conventional breeding a plateau has been achieved for the level of sucrose accumulation in the culm. Contrary to this, it has been also opined that the genetic stocks being used possessed narrow genetic base[13], but many researchers still believe that significant potential exists for further gains in sucrose content without affecting other economical traits[3,14]. A noteworthy work[3] where the level of sugar content has been doubled through the novel incorporation of a bacterial gene (sucrose isomerase, SI) which produces sucrose isomer isomaltulose that has the added advantage of its slow metabolizing ability in human intestine, clearly showed an alternate pathway of addressing such issue. Enhanced sugar accumulation in sugarbooster line (transgenic bearing SI gene) was accompanied with increased rate of photosynthesis, electron transport reflecting photosynthetic efficiency, sugar transport and most importantly sink strength. Doubling the total sugar content in mature internodes of an elite high-sugar cultivar eliminates osmotic limits and osmotic sensing as primary constraints behind the previous concentration ceiling in sugarcane[2,13]. Utilizing EST resources and gene expression analysis during culm development, provided valuable information, but could not help identify the factors regulating sucrose storage in the culm. Papini-Terzi *et al.*[11] identified the differentially expressing genes in genotypes varying for Brix (sugar) through a study of cDNA microarrays, and many genes have shown correspondence with protein kinases and transcription factors that may regulate sucrose accumulation.

In the last five years, research has been mainly focused on source–sink relationships, so as to increase the sucrose content by elucidating the genes and enzymes in sugarcane leaves that were responsive to changes in the ratio of source–sink. Two schools of thought (N. G. Inman-Bamber and A. J. McCormick) are clearly visible and interesting information pertaining to source–sink relationships has been generated as far as increased sucrose content in the culm is concerned. One group suggests pursued research and development to be continued by targeting leaf extension rate, photosynthesis, water regime and temperature management vis-à-vis their manipulation in improving sucrose content[4,15,16], whereas the other group suggests doing research on regulation of expression of gene(s) associated with carbohydrate metabolism vis-à-vis overall plant physiological responses that might detect mechanisms that mediate the relationships between source and sink tissues[17,18]. Novel experimentations like partial leaf shading, partial defoliation where single leaf (source) was capable of maintaining a nominal supply of carbon based on the demand from the sink tissues (mostly culm) have been performed. Further studies indicated significant increase in photosynthetic rate of the sole source leaf had negative correlation with sucrose level in immature culm tissues providing good evidence of sink regulation of photosynthesis in sugarcane. However, the study lacked the molecular mechanism that mediates communication between source and sink in sugarcane. Gene regulation during culm development and sucrose accumulation, identification of genes differentially expressing in high and low sugar-accumulating genotypes as well as those genotypes showing contrasting behaviours towards abiotic stress, especially drought may not provide the exact nature of the feedback mechanism that is operating between the source and sink. Therefore, the focus should be to study the physiological responses along with expression of genes associated with carbohydrate metabolism so as to discern the regulatory mechanism and its interrelationships between source and sink tissues[19]. Some initial attempts have been made by McCormick *et al.*[17] to study the changes in the behaviour of expression of carbohydrate metabolism-related genes that are associated with source–sink perturbation in sugarcane.

In view of the complexity of sucrose metabolism at physiological, biochemical and genetic levels, the most important issues that need to be addressed are: identifying transcription factors regulating accumulation of sucrose; feedback inhibition studies so as to underpin the modulation of source–sink relationships; biophysiological changes along with leaf gene expression patterns; in case sink strength controls the source activity, then how the signal is being transported and the potential signal molecules, besides hexoses (so far reported) and finally, how genes of other physio-biochemical responses like drought, ABA signalling and genes belonging to lignin biosynthesis, cell-wall metabolism and aquaporins exert regulatory control over sucrose accumulation. So far sucrose-associated genes have been found directly responsive to short-term sucrose stimuli, thus confirming their role in sugar-related pathways[11]. Finally, if genes responsible for mediating the communication between source and sink tissues in sugarcane are cloned and characterized, transgenic research will pave the way to use them in sugarcane improvement programmes, especially for augmenting sucrose content. Such research results will also focus on the important players of the sugar signal transduction pathways (Figure 1). Major research should be oriented towards deciphering how the sink acts to regulate the source activity; it will provide additional potential targets to researchers to manipulate the grey areas in sucrose–synthesis–accumulation pathway, which has been an enigma for sugarcane researchers since the last five decades. Such information will have larger impact on other C_4 crops as sink controls the source activity and thus the signal feedback system reporting sink sufficiency and regulating source activity as the prime target for genetic manipulation. It will finally culminate into a significant modification as plant genomes translate into changes in growth and development in a range of environments.

In the present scenario, the following points need to be addressed so as to enhance the level of sucrose in sugarcane: addressing the feedback inhibition system of source–sink to know whether the sink and its perturbation will pave the way to identify the crucial factor that controls the level of sucrose to be accumulated in culm. If the source and sink work perfectly as supply and demand scenario, how and when does demand control the supply? If the sink controls the source, then what is the significance of excess source available with the crop (extra unnecessary load)? Does the basic and fundamental difference in cane yield as observed in subtropical and tropical parts of the country have any relevance

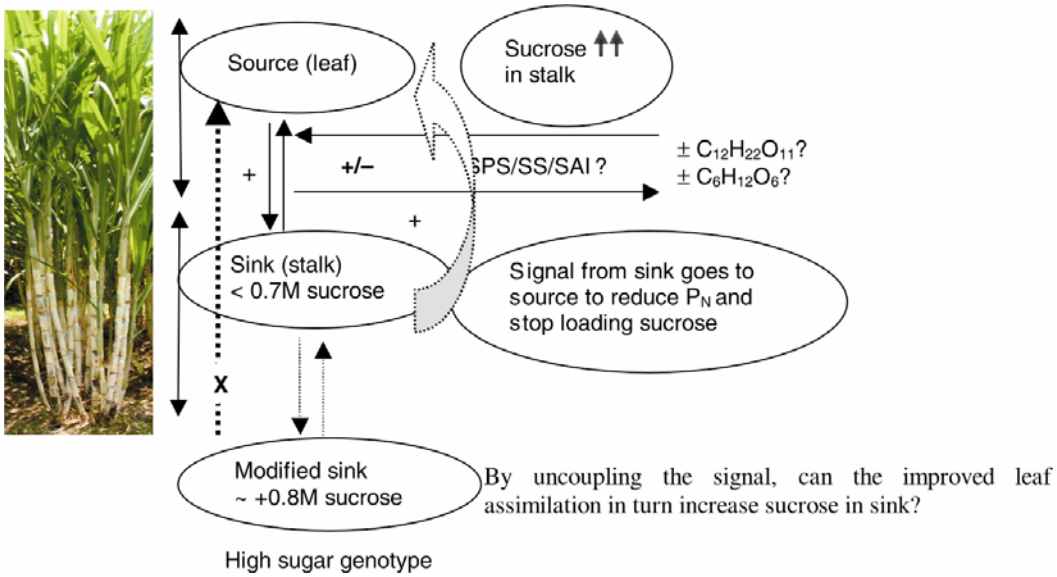

Figure 1. Source–sink communication plays an important role in accumulation of sucrose in sugarcane culms.

with source–sink relationship, or it is totally a weather-controlled mechanism? Will sugarbooster line (transgenic plant able to accumulate sucrose along with isomaltulose) deliver new insights into the mechanism by which plants regulate sugar accumulation (a pivotal question in plant biology)? Finally, how elevated levels of CO_2/high temperature combination increase the sucrose in juice and yield of the crop[20] will lead to address the source–sink scenario in sugarcane? Further, the potential merit of cellulosic biofuels from sugarcane baggase vis-à-vis increased sucrose content has invigorated research into this crop over other potential biofuel-producing crops, viz. *Miscanthus* and *Panicum*. The gradual change in environmental matrix especially elevated levels of CO_2 and high temperature, are bound to impact sucrose synthesis–accumulation dynamics in sugarcane. It is imperative that new resources and tools are judiciously utilized to unravel the physio-biochemical processes of sugarcane plant, especially sucrose metabolism.

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