TRANSCRIPTOMIC ANALYSIS OF SOURCE-SINK DYNAMICS ASSOCIATED WITH SUGAR ACCUMULATION IN SUGARCANE

SUMMARY

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SUMMARY

Sucrose forms the high-value, primary product of interest from sugarcane. The sucrose content of culm is largely determined by its stage of development. The culm is known to mature upwards from bottom, with the sucrose gradient following the same trend. Thus, broadly, the lower, more mature internodes amass more sucrose while the upper, immature ones house bulk of hexoses, with the gradient evening out with advancing maturity. Nonetheless, till date, the sucrose yield realized is much lesser than the physiological maxim possible from sugarcane. This issue of low recovery has mainly been made up for, by increasing the cane yield. However, the increasing scarcity of cultivable land plus the towering costs involved in cane transport and processing, make 'enhancement of sucrose concentration of culm', a more viable target for improving sucrose productivity. Hence, a comprehensive understanding of the mechanism of sucrose accumulation in the culm is essential, in order to design manipulation strategies to make the most of its actual sucrose-accumulating potential.

In most higher plants, the photosynthetically active leaves serve as 'source' for carbon and energy. Particularly in sugarcane, the culm serves as reservoir or sink for the photoassimilate that is transported as sucrose from source to the sink. The transported sucrose is either immediately utilized for growth, developmental and metabolic processes or immobilized for storage. Such consumption of sucrose causes a high sink demand to develop, thus resulting in the extraordinary levels of sucrose observed in sugarcane. Hence, the communication between source and sink can be visualized in the light of a supply-demand concept wherein the source leaves synthesize and 'supply' sucrose in response to the sucrose 'demand' that is generated in the sink. The crop yield can perhaps be enhanced by either improving source capacity (to produce photoassimilate) or by increasing consumption of photoassimilates by sink (thereby enhancing sink demand). However, since the inflow of sucrose in sugarcane is primarily governed at the level of sink, sucrose accumulation in the culm is perhaps mainly sink-regulated.

Sugar-sensitive systems are said to operate in source leaves causing leaf photosynthetic rate to be modified by sugar molecules viz. sucrose and hexose, indicating their key role in altering the carbon distribution between source and sink.

The activity of various sugar metabolizing enzymes and expression of related gene transcripts, is perhaps adapted according to the sink demand. Hence, studying the modulation of expression of sucrose metabolism-related genes under conditions of modified sink demand may help better decipher the source-sink relationship and consequently sucrose accumulation in the culm. Some earlier efforts made in this direction have brought to light variation in the expression of some carbohydrate metabolism-related genes in answer to source/sink manipulation in sugarcane. Defoliation study in sugarcane pointed to escalation in photosynthetic rate of the sole source leaf in response to drop in sucrose level in culm, emphasizing that photosynthesis is regulated at the level of sink. However, the studies so far have largely lacked in providing a molecular basis to the complex mechanism underlying source-sink dynamics. This necessitates the scrutiny of sucrose metabolism (synthesis and storage) at source-sink level, in order to better understand sucrose accumulation in sugarcane.

An array of factors governs sucrose synthesis and transport leading to sucrose accumulation in the sugarcane culm. Several works (as discussed in review of literature) have brought to light genes/enzymes involved in sugar synthesis and metabolism, those that facilitate effective sucrose transport and that govern partitioning of sugar into subcellular compartments. Exploring the interplay of all such factors through perturbation of source-sink communication and the study of consequential changes therein, at physiological, biochemical and molecular level, over the sucrose accumulation phase, will facilitate better understanding of the source-sink dynamics that underlies sucrose accumulation process.

Thus, the following objectives were set:

- To develop a biophysiological insight into the source (leaf)-sink (culm) communication of sugarcane, leading to sucrose accumulation in the culm.
- To study the effects of modulation of source-sink dynamics, on sucrose accumulation in the culm.
- To analyse changes in expression of genes, in response to source-sink perturbation and identify novel genes.

The present work provides a more holistic picture about the interplay of factors (morphological, biochemical and molecular) that determine sucrose accumulation, providing connecting links to earlier studies. To the best of my knowledge, this is a first-of-its-kind work where sink behaviour has been tracked and analysed in relation to source activity under the effect of gibberellic acid (GA_3) treatment. Gibberellins have long been known to encourage elongation and division of cells, thus providing more room for assimilate accumulation, thereby improving sink's capacity to accommodate sucrose. In the present pursuit, GA_3 spray was employed to delay saturation of sink by enhancing sink strength and sink demand, perhaps, thereby facilitating greater sucrose accumulation. A comparative was drawn between difference in responses of a high sugar (CoJ64) and a low sugar accumulating variety (BO91) to elucidate the factors that facilitate better sucrose accumulation. The differential pattern of various morphological (viz. plant height, internode length, cell size) and biochemical (RS, sucrose, pol%, brix%, NR, chlorophyll, invertase and SPS activity) parameters, in control (untreated) and treated canes was analysed in response to such manipulation of sink capacity. Also, the difference in expression of various sucrose metabolizing enzymes *viz*. soluble acid invertase (SAI), cell wall invertase (CWI), neutral invertase (NI), sucrose synthase (SuSy) and sucrose phosphate synthase (SPS), which are considered to play important role in sucrose accumulation in sugarcane, was analysed. Additionally, expression of other related molecular determinants (viz. PEPC, SUT), was explored under controlled and GA_3-induced perturbation to ascertain the effect of GA_3 on sucrose metabolism and transport, at molecular level.

SEM visualization revealed larger cell size in the GA_3 treated internodal samples of both varieties, as compared to that in control. This indicates the role of GA_3 in enhancing sink size and sink capacity, providing more room for sucrose accumulation. In general, greater chlorophyll content was observed in the GA_3 treated leaf defending the role of GA_3 in facilitating higher photosynthetic rate to meet higher sink demand developed under GA_3 effect. Higher NR activity was seen in the GA_3 sprayed leaf during initial phase pointing to increased N uptake and utilization, in order to support increased sink demand under GA_3 effect.

The GA_3 treatment produced most conspicuous change, 30 days after spraying (30 DAS), wherein the reducing sugar (RS) level significantly escalated, especially in the

upper internodes of GA_3 treated canes. This perhaps points to increased sink strength and in turn better sink demand to attract more assimilate. The difference was more drastic in case of BO91, than in CoJ64, suggesting more scope for GA_3-induced manipulation of sink capacity, perhaps due to its late maturing nature. The sharp rise in RS levels and corresponding fall in sucrose content was validated by a parallel higher invertase activity observed in GA_3 treated samples. Higher invertase activity in agreement with higher RS values observed in BO91 can be extrapolated to better sink strength, as compared to CoJ64. Concurrently, lower RS and greater sucrose levels were noted in the GA_3 treated LTM leaf, indicating greater conversion of RS to sucrose, to answer the high sink demand developed. These observations can be extrapolated to interpret that GA_3 perhaps maintains a prolonged state of immaturity in the culm tissue, thereby resulting in better sink strength. Noticeably higher RS values were observed up to 60 DAS, particularly in BO91, beyond which the sucrose levels rose with analogously high brix%, pol% values. Even as the GA_3 effect gradually waned and maturation advanced, the GA_3 treated canes exhibited evidently higher sucrose levels, substantiating the envisaged increase in sink capacity. Higher SPS activity was observed in the GA_3 treated internodal samples of CoJ64 and BO91, up to 60 and 120 DAS respectively, In congruence with RS values, invertase activity declined over time. As a result, higher final sucrose concentrations were obtained in GA_3 treated BO91, affirming the possibility that GA_3 treatment can bring about improvement in sucrose accumulation in culm.

The end-point and real time-PCR based differential expression data was in congruence with these biochemical findings. Evidently higher expression of SAI was obtained especially 30 DAS, pointing to relative immaturity or delayed onset of maturity due to GA_3 effect. The higher CWI expression obtained in GA_3 treated plants, perhaps signifies higher apoplastic unloading of sucrose, thereby affirming greater sucrose uptake under GA_3 influence. The particularly higher SPS expression observed in GA_3 treated BO91 cane, compared to control, perhaps explains its high sucrose yield at the end of study. Correspondingly, heightened SUT expression and coherent dip in sucrose levels observed in GA_3 treated LTM leaf, also corroborate increased sucrose transportation. GA_3 treated LTM leaf also displayed higher PEPC expression, than in control, in congruence with high RS levels recorded, validating greater photosynthetic rate in response to high sink demand. GA_3 was also found to

stimulate marked rise in SPS expression in GA_3 treated LTM leaf depicting greater sucrose synthesis in response to the higher sink demand. At the end of maturation phase, inhibition of phloem loading due to decreased sink demand perhaps signalled sucrose sufficiency, causing diminished SPS expression in leaf.

BO91 displayed a more promising response to GA_3 treatment, with final peak sucrose values as high as (40.54%-41.6%) recorded in GA_3 treated BO91 canes, compared to (30.44%-38.8%) in control ones. In view of the above analysis, the transcriptome sequencing and analysis of control and GA_3 treated top internodal samples (C_T and G_T respectively) was carried out *de novo*. The transcripts derived from C_T and G_T, were screened on the basis of read count and those exhibiting substantial read count difference, of two fold and more, were sieved out to obtain 558 transcripts. BLAST2GO was employed to determine the functional annotation of these transcripts. BLASTing revealed that top hits of most transcripts shared homology with *Sorghum bicolor*, followed by *Zea mays*, *Sertaria italica* and *Saccharum* hybrid cultivar R570. From among these, 26 differentially expressing transcripts were selected on the basis of assigned function and homology. Real-time primers were designed using the online IDT tool, in order to validate the RNA-seq results. Out of the 26, 13 primers were employed to ascertain their differential expression among C_T and G_T, over the maturation phase. In this pursuit, a valid transcript showing 95% homology with a probable sugar phosphate/phosphate translocator At1g06470 of *Sorghum bicolor*, was identified. Its augmented expression in the GA_3 treated sample affirmed the active role of this transcript in sugar transport. The quest also fished out transcripts homologous to vital genes from close homologs viz. mRNA of fructose-1,6-bisphosphatase of *Saccharum* hybrid cultivar H65-7052, UTP-glucose-1-phosphate uridylyltransferase of *Sorghum bicolor,* cytochrome b-c1 complex subunit of *Sorghum bicolor*, WAT1-related protein of *Sorghum bicolor,* MYR1 of *Sorghum bicolor*, translation initiation factor5A (elF5A) mRNA (complete cds) of *Saccharum* hybrid cultivar ROC22, scarecrow-like protein of *Sorghum bicolor*, V-ATPases (vacuolar-type H+ ATPase) of *Sorghum bicolor*, glycosyltransferase. Most transcripts exhibited expression in keeping with the RNA-seq results except one displaying homology to a sucrose transporter protein of *Saccharum* hybrid cultivar GT28 which showed downregulated expression throughout the maturation phase. A transcript was

also found to display 97% homology with mRNA for a predicted uncharacterized protein ycf39 (LOC8058013) of *Sorghum bicolor*.

Overall, the key outcome of this study was that GA_3-treated BO91 cane displayed significantly higher final sucrose concentration, compared to control cane. Though, similar observation was also made in case of GA_3-treated CoJ64, however the high sucrose levels could not be sustained perhaps due to onset of inversion in the early maturing variety.

The key findings that can be concluded from this study are:

- GA_3 treatment induced an increase in cell size denoting increase in sink size and sink capacity to accommodate more sucrose
- GA_3 treatment perhaps prolonged the state of immaturity in cane thereby stimulating invertase (SAI) activity and consequently reducing sugar content of the culm, thereby depicting increase in sink strength and heightened sink demand to attract more assimilate
- The escalated expression of various genes viz. CWI (pointing to apoplastic unloading), SPS (bringing about sucrose synthesis), PEPC (depicting photosynthetic rate), SUT (involved in sucrose transportation), all lent support to greater sucrose accumulation in GA_3 treated cane
- Chlorophyll content increased in response to GA_3 treatment to support increased photosynthesis for production of more photoassimilate to meet the high sink demand; also qualified by increase in reducing sugar in GA_3 treated leaf samples
- The RS, sucrose values validated a prolonged state of immaturity induced by GA_3 in the late maturing BO91(higher RS even at 60 DAS) while GA_3 treatment was effective for a shorter span in the early maturing CoJ64, thus corroborating the higher sucrose accumulated in GA_3 treated BO91
- Transcriptomic analysis identified several transcripts exhibiting significant differential expression under the effect of GA_3 spray. Transcripts identified as homologous to sugar phosphate/phosphate translocator, UTP-glucose-1-phosphate uridylyltransferase, V-ATPases (vacuolar-type H+ ATPase) were

found to be perhaps correlated to the high sucrose content observed in GA_3 treated cane. A differentially expressing transcript found homologous to mRNA of an uncharacterized protein and characterized as hydrolase (using online resource) may prove to be novel stimulant of sucrose accumulation process.

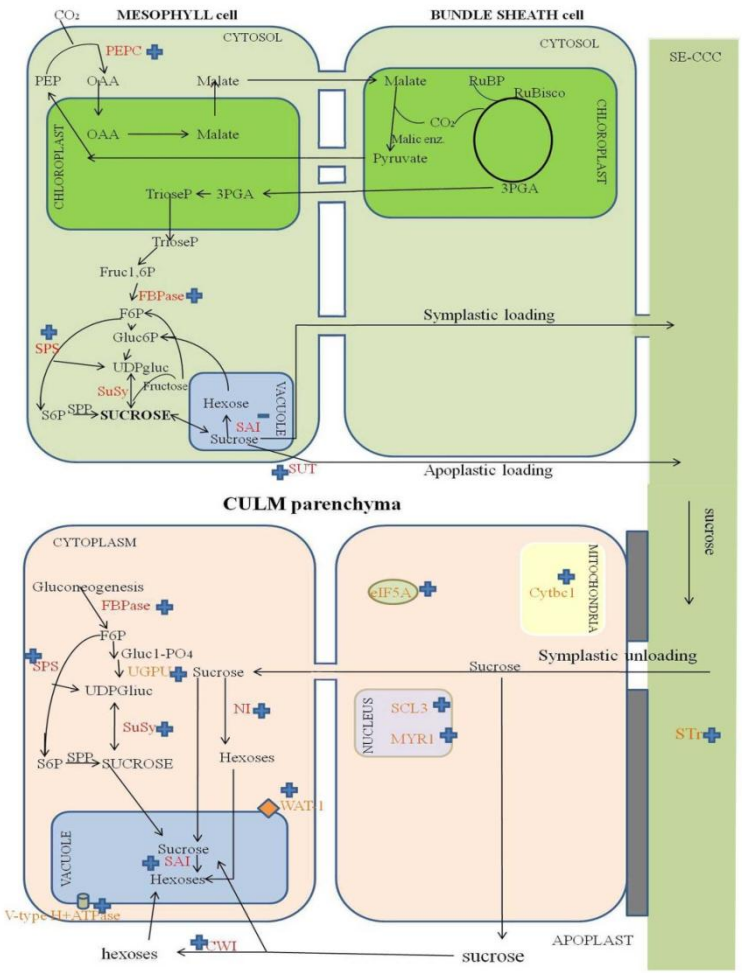

Figure: Nutshell diagram of some molecular determinants of high sucrose accumulation in culm (as learnt by comparison of control and GA_3 treated samples, a month after GA_3 spray) (adapted from Watt *et al*., 2014) The well-known determinants conventionally used in studies include SAI, CWI, NI, SuSy, SPS, FBPase, SUT, PEPC while the novel stimulants ascertained in the transcriptomic study include sucrose translocator (STr) , UTP-glucose-1-PO_4 uridylyltransferase (UGPU), cytochrome b-c1 complex subunit (cytbc1), WAT1, V-type H^+ ATPase, transcription factors MYR1and SCL3, elongation factor eIF5A

\+ denotes up regulated expression and - denotes down regulated expression