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VIAS DE PERCEPÇÃO E SINALIZAÇÃO DE AÇÚCARES DURANTE O CRESCIMENTO E DESENVOLVIMENTO DA CANA-DE-AÇÚCAR (*Saccharum* spp.)

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RESUMO

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O bioetanol derivado da cana-de-açúcar (*Saccharum* spp) é uma fonte de energia sustentável que contribui para a mitigação das emissões de carbono. Entender como a cana-de-açúcar coordena o equilíbrio entre assimilação, alocação e uso de carbono (C) é crucial para aumentar a produção da cultura sem aumentar as áreas plantadas. Uma rede de sinalização complexa capaz de detectar os níveis de C e energia e integrá-los ao crescimento e desenvolvimento das plantas inclui os seguintes componentes: *hexokinase* (HXK), *trehalose-6-phosphate* (T6P), *the target of rapamycin complex 1* (TORC1), e *sucrose-non-fermenting related protein kinase 1* (SnRK1). Todas essas vias de sinalização regulam e são reguladas por açúcares e orquestram o fluxo de C. No entanto, ainda não está claro como isso ocorre, especialmente para a cana-de-açúcar, cujo genoma é poliplóide e altamente complexo. Assim, o objetivo principal desta tese foi identificar os genes dos sensores de açúcares mencionados acima em cana-de-açúcar (variedade SP80-3280). Para isso, sequências das proteínas de HXK, TORC1, SnRK1 e T6P foram identificadas e caracterizadas *in silico* em cana-de-açúcar, para as quais apenas conjuntos genômicos incompletos estão disponíveis. Resumidamente, sequências de genes ortólogos de espécies modelo e sete bancos de genoma e transcriptoma de cana-de-açúcar foram utilizados para inferência filogenética e identificação de domínios funcionais de proteínas.

Além disso, plantas de cana-de-açúcar cultivadas no campo ao longo do ciclo de desenvolvimento (01, 03, 06 e 12 meses) foram utilizadas para análises de aminoácidos e poliaminas, uma vez que as vias de sinalização que controlam o metabolismo do C também possuem um *cross-talk* com essas vias. Paralelarmente, a atividade de HXK foi quantificada. Muitas sequências putativas de comprimento total de todas as vias foram recuperadas e analisadas em relação a todos os domínios conservadores de cada alvo. Especificamente para TORC1, este trabalho é pioneiro na recuperação de sequências de todas as proteínas membros do complexo (TOR, RAPTOR e LST8). Com relação a via da

T6P, foram descobertas diferenças essenciais nas sequências catalíticas (forma o metabólito T6P) e reguladoras das TPSs da cana-de-açúcar, como algumas mutações de resíduos implicadas na perda da atividade enzimática. O primeiro mês de desenvolvimento da cana-de-açúcar foi marcado pela maior atividade de HXK nos colmos e maior abundância de aminoácidos totais neste tecido. Para as poliaminas totais, há maior abundância na folha em comparação com os colmos. A putrescina foi a poliamina mais abundante na folha no mês 01, possivelmente atuando como promotor de crescimento. Embora o mecanismo completo de sensores e sinalização de açúcar na cana-de-açúcar não tenha sido totalmente elucidado, as sequências recuperadas e os novos dados obtidos no experimento de campo serviram para construir um painel contendo todos genes selecionados. Com essa análise, será possível obter informações básicas (como valor de expressão) tanto para elucidar o mecanismo de ação dos alvos quanto para aplicações biotecnológicas, como melhorar o acúmulo de sacarose ou melhorar o desempenho da planta frente aos estresses ambientais aos quais está exposta.

Palavras-chave: Carboidratos. Cana-de-açúcar. Sensores de açúcares.

ABSTRACT

DE OLIVEIRA, L.P. **Sugar perception and signaling pathways during sugarcane development (*Saccharum* spp).** 2022. 160 p. Ph. D. these (Biotechnology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2022.

The bioethanol derived from sugarcane (*Saccharum* spp) is a sustainable alternative energy source contributing to the mitigation of carbon emissions. Understanding how sugarcane coordinates the balance among carbon (C) assimilation, allocation, and usage is crucial to increasing crop production without expanding the planted areas. A complex signaling network capable of sensing C and energy levels and integrating them with plant growth and development includes the following players: hexokinase (HXK), trehalose-6-phosphate (T6P), the target of rapamycin complex 1 (TORC1), and sucrose-non-fermenting related protein kinase 1 (SnRK1). All these signaling pathways regulate and are regulated by sugars and orchestrate the C flux. However, it remains unclear how this occurs, especially for sugarcane, whose genome is polyploid and highly complex. Thus, the main objective of this thesis was to identify genes of the sugar sensors mentioned above in sugarcane (variety SP80-3280). For this, sequences of HXK, TORC1, SnRK1, and T6P metabolizing enzymes were identified and characterized *in silico* in sugarcane, for which only incomplete genome assemblies are available. Briefly, sequences of orthologous genes from model species and seven sugarcane genome and transcriptome databases were used for phylogenetic inference and identification of functional protein domains.

Additionally, the metabolite profile of sugarcane plants grown in the field throughout the developmental cycle (01, 03, 06, and 12 months) was expanded to include amino acids and polyamines since the signaling pathways that control C metabolism also have a cross-talk with these pathways. Moreover, the activity of HXK has been quantified. Many putative full-length sequences from all pathways were recovered and analyzed concerning all conservative domains of each target. Specifically for TORC1, this work is the pioneer in recovering sequences from all protein members of the complex (TOR, RAPTOR, and LST8). Concerning the T6P pathway, essential differences in TPS catalytic (forms the T6P metabolite) and regulatory sequences of sugarcane were discovered, such as some residue mutations implicated in the loss of enzyme activity. The first

month of sugarcane development was marked by the highest HXK activity in the culms and a greater abundance of total amino acids in this tissue. For total polyamines, there is a greater abundance in the leaf compared with culms. Putrescine was the most abundant polyamine in the leaf at month 01, possibly acting as a growth promoter. Even though the complete mechanism of sugar sensors and signaling in sugarcane has not been fully elucidated, the sequences recovered and the new data obtained from the field experiment served to build a panel containing selected essential genes. With this analysis, it will be possible to get basic information both to elucidate the mechanism of action of the targets and for biotechnological applications, such as improving sucrose accumulation or improving plant performance in the face of environmental stresses to which it is exposed.

Keywords: Carbohydrates. Sugarcane. Sugar sensing.

GENERAL INTRODUCTION

General Introduction

According to the United Nations (UN), in 2050, the world population will increase from currently 7.7 billion to 9.7 billion people (United Nations Population, 2019), significantly raising the demand for land use, food, and energy sources to maintain human activities. If no action is taken, this will imply an increase in carbon dioxide (CO_2) and greenhouse gas (GHG) emission levels, resulting in losses in agriculture, particularly in food and energy production. The transport sector alone is responsible for producing 7.0 of Global GHG emissions ($GtCO_{2eq}$), including non-CO_2 gases. Currently, transport has the highest reliance on fossil fuels and accounts for 37% of CO_2 production from end-use sectors (International Energy Agency, 2021). In this scenario, the production of biofuels has emerged as a viable ecological substitute to avoid emissions (Beard et al., 2021). It has been identified as an efficient alternative to mitigate global climate changes (Buckeridge et al., 2012).

The use of biomass to produce energy reduces the dependence on fossil fuels and has a positive impact on many environmental issues, helping to minimize the production of GHG (De Souza et al., 2014). However, to be considered an ideal energy-producing crop, some attributes are needed: fast growth, high yield, reduced inputs (e.g., water and nutrients), low processing cost, and increased positive energy balance (Waclawovsky et al., 2010). Sugarcane (*Saccharum* spp) has all these characteristics (Mohan; Easterling; Yau, 2021), with Brazil being the world's largest producer and one of the leading suppliers of sugar and bioethanol worldwide (Cursi et al., 2022). The industrial process of these two commodities is integrated, so that competition between food (sugar) and fuel production is minimized, differently from other crops like maize and sugarbeet (Waclawovsky et al., 2010). Due to its incomparable ability to produce biomass, sugarcane has been increasingly used as a sustainable resource of energy since lignocellulosic residues (bagasse and straw) have the potential to increase crop productivity without alterations in the planted areas, respecting demarked agroecological zoning (Buckeridge; Santos; Souza, 2014; Jaiswal et al., 2017).

The industrial strategy consists of fermentation of the stored soluble sucrose to produce first-generation (1G) bioethanol, representing only one-third of the sugarcane biomass (Buckeridge; Santos; Souza, 2014). The other two-

thirds are composed of lignocellulosic residues that can be used to produce the second-generation (2G) bioethanol. The vinasse, the remaining product of ethanol distillation, has the potential to produce biogas (Cursi et al., 2022). These multiple strategies of industrial sugarcane usage associated with cellulosic ethanol production can contribute to greater offsetting of carbon (C) emissions (Jaiswal et al., 2017).

Despite these advantages, bioethanol is still not economically accepted in the energy market compared to fossil fuels (Merritt; Barragán-Ocaña, 2021). One alternative to stimulate the use of bioethanol would induce policymakers to pay attention to the market and encourage the consumption of economically viable and environmentally sound energy supply alternatives to fossil fuels (Merritt; Barragán-Ocaña, 2021). To deal with these factors, in addition to political issues, several public, and private Brazilian institutions, including the Agronomic Institute of Campinas (IAC), Brazilian Agricultural Research Corporation (EMBRAPA), Inter-University Network for the Development of the Sugar-Alcohol Sector (RIDESA), and Sugarcane Technology Center (CTC), have contributed to increasing crop productivity through genetic improvement of sugarcane via conventional breeding. Cane yields or sugar content have grown along with the ratooning ability, disease resistance, and abiotic stress tolerance (Jackson, 2005; Ming et al., 2005; Scortecci et al., 2012). However, the average Brazilian production is still far below the theoretical potential of the crop and lower than that of some other countries (Cursi et al., 2022). Some reasons for this are that the development of new sugarcane varieties by conventional breeding is very lengthy (10 – 15 years). Its genome has not been wholly sequenced because it is complex, interspecific, polyploid, and extensively aneuploid (Thirugnanasambandam; Hoang; Henry, 2018). These complex genetic characteristics challenge manipulating multiple genes or intricate metabolic pathways, the integration among distinct quantitative traits loci, resulting in low heritability of economically essential characters.

Incorporating new knowledge about physiological, biochemical, genetic, and phenotyping processes, mainly associated with marker genes, is important to fill the gaps that can contribute to the development of new strategies in sugarcane (Calderan-Rodrigues et al., 2021). Thus, one of the greater biotechnological potentials that aim to increase sucrose in the culm depends

mainly on the knowledge of the pathways involved in the partition of photosynthetic C assimilated and the source (mature leaves) and sink (non-photosynthetic tissues) relationship, which is one of the main limiting factors for storage this disaccharide in sugarcane (Calderan-Rodrigues et al., 2021).

In the source tissues, the availability of resources relies on photosynthesis and the production of sugars. Sucrose is the main product and can be immediately consumed for cellular functions or transported to other tissues to sustain growth and development. Concomitantly, starch accumulates in leaves during the day and is degraded at night to produce sucrose (Smith; Stitt, 2007). This is a well-coordinated process that exchanges internal signals, influenced by environmental factors perceived by sugar sensors and signals (Paul; Foyer, 2001; Smith; Stitt, 2007; Martins et al., 2013). The concept of sugars as central signaling molecules is relatively recent (Rolland; Baena-Gonzalez; Sheen, 2006). To illustrate, Ferrari and collaborators (2013) showed an example of sugar, the oligogalacturonides (OGs), oligomers of alpha-1,4-linked galacturonosyl residues that are integral components of the cell wall and also act as a signaling molecule. Under biotic stress conditions, this saccharide can be released from the cell wall by enzymes activated by fungal growth or by mechanical damage and acts as a signaling molecule to trigger a defense response in the individual plant cell and surrounding tissues (Ferrari et al., 2013). This integrative plant perception and management of sugar levels are crucial for their development, which leads to a unique C signature in plant cells, tissues, and organs (Li; Sheen, 2016; Smith; Stitt, 2007).

Sugar sensing pathways in plants

Plants have an outstanding ability to perceive and respond to resource availability, which was an essential adaptive strategy to bypass their sessile characteristic and efficiently maintain energy equilibrium (Rolland; Baena-Gonzalez; Sheen, 2006; Lastdrager; Hanson; Smeekens, 2014). For this, the sugars produced by photosynthesis do not function only as substrates for metabolic reactions, but they strikingly orchestrate internal signal transducer mechanisms (Paul; Foyer, 2001). Sugars are the best-understood example of metabolites that have a crucial role in allosteric effectors and feedback inhibitors of various metabolic enzymes. They connect dynamic processes such as their

production, transport, consumption, and storage, characteristics that are linked to cellular physiology, organ identity, and developmental stages. Therefore, the integrative plant perception and management of sugar levels could serve as a control mechanism to integrate external environmental factors, nutrient homeostasis, developmental programs, and stress response by controlling anabolic and catabolic processes (Smith; Stitt, 2007; Li; Sheen, 2016).

Sugar sensing genes in plants are a part of a cellular adjustment that perceives nutrient availability by adjusting growth and biosynthetic activities (Koch, 1996). This process occurs due to the ability to detect changes in sugar levels, such as glucose, fructose, sucrose, and trehalose-6-phosphate (T6P), and respond through a complex signaling network to gene expression and protein activity regulations to deal with the imposed changes (Martínez-Noël; Tognetti, 2018). Some genes are little affected; others are significantly activated and repressed. Furthermore, in plants, the sugar-regulated expression also provides a mechanism to control the distribution of resources among tissues and organs (Koch, 1996).

One well-known mechanism to respond to sugar levels is phosphorylation and dephosphorylation through protein kinases and phosphatases, which can activate or deactivate their targets (Noël; Tognetti; Pontis, 2001). These proteins have a highly conserved catalytic domain but a specific regulatory domain that defines the protein kinase type and its exact role in metabolism (Heidrich et al., 2010). Phosphorylation of targets can have diverse effects: it can result in enzyme activation and/or inhibition, the formation of recognition sites for recruitment of other proteins, and transitions in protein state from order to disorder or vice versa (Johnson; Lewis, 2001). Some sugar sensors are protein kinases that have already been described like hexokinase (HXK), sucrose non-fermenting related protein kinase 1 (SnRK1), and the Target of Rapamycin (TOR) that composes the multiprotein complex kinase 1 (TORC1).

High glucose levels have been reported as an activator of TORC1, which induces various processes like glycolysis and biosynthesis of sucrose and starch (Dobrenel et al., 2016). On the other hand, low sucrose levels can activate SnRK1, causing a decrease in energy consumption and stimulating energy-producing catabolic processes (Figueroa; Lunn, 2016). Furthermore, sucrose can also affect the levels of the T6P, which is an essential metabolite in plants and

also can modify the metabolism at different levels (Lunn, 2007; Martins et al., 2013; Lunn et al., 2014; Yadav et al., 2014)

The following sections will describe these plant sugar sensing and signaling pathways and explore their most notable roles.

Hexokinase (HXK)

In sink organs, sucrose is hydrolyzed into glucose and fructose by invertase or cleaved into UDP-glucose and fructose by sucrose synthase (Wan et al., 2018). These hexoses are then phosphorylated by HXK and fructokinase (FRK). In plants, glucose can be phosphorylated only by HXK, whereas fructose can be phosphorylated by either HXK or FRK. However, the affinity of HXK for glucose is twice as higher than that for fructose due to the better substrate stabilization by the amino acid residues in the catalytic site (Granot et al., 2014).

For *Arabidopsis thaliana*, HXK proteins play dual functions, one enzymatic (of phosphorylation, mentioned above) and the other as glucose sensors by integrating nutrient, light, and hormone signaling (Moore et al., 2003). HXK has been described in some plants as a multigene family (Moore et al., 2003; Zhang et al., 2014). In Arabidopsis, only three HXKs are catalytically active (HXK1, HXK2, and HXK3). In contrast, the others are known as hexokinase-like (HXL) (HXK-Like 1, HXK-Like 2, and HXK-Like 3) and cannot phosphorylate glucose due to a series of changes in their primary sequences. Some functions have been described for HXL. For example, the HXK-Like 1 acts as a negative plant growth regulator that affects seedling growth responses to glucose and auxin (Karve; Moore, 2009)

Among all six HXKs in Arabidopsis, only three are sugar sensors: HXK1, HXK3, and HXK-Like 1 (Aguilera-Alvarado et al., 2019). This role could be related to the high affinity of HXK for glucose (Feng et al., 2015), which was recently identified by structural crystallographic analysis of two HXKs, the HXK1 and a catalytically inactive version of *A. thaliana* (Aguilera-Alvarado; Sánchez-Nieto, 2017). Nevertheless, it is still not elucidated how the conformational movement encouraged by glucose binding in the HXK promulgates the glucose abundance signal (Aguilera-Alvarado; Sánchez-Nieto, 2017).

The *A. thaliana* mutant *gin2-1* (a null mutant of *HXK1*) is insensitive to high glucose concentrations and presents many phenotypic changes, including

reduced shoot and root growth, decreased leaf expansion, late flowering, increased apical dominance, reduced auxin sensitivity, improved cytokinin sensitivity, and changes in transcript levels of several target genes (Moore et al., 2003). Its sensor activity was further confirmed by the complementation with a catalytically inactive HXK1 protein that did not produce Glc6P but could transduce the glucose status signal restoring glucose sensitivity (Moore et al., 2003). These works proved that when *AtHXK1* acts as a sensor, its catalytic activity is not essential. However, the precise mechanism responsible for this activity is not clearly understood (Rodríguez-Saavedra et al., 2021).

Although all HXKs known display two domains, a large (C-terminal, catalytic) and a small (N-terminal, regulatory) domain, their sequences have many differences. For example, the AtHXK-Like 1 and AtHXK-Like 2 have 6-10 amino acid insertions or deletions at the adenosine binding domain (N-terminal), in contrast to HXK-Like 3 (Karve et al., 2008). Furthermore, HXK can have different subcellular localizations such as mitochondria, the Golgi complex, and chloroplasts (Zhou et al., 2014). This feature, associated with other structural differences (e.g., introns and exons disposition), is reflected in their function. For these reasons, HXKs are considered moonlighting proteins, defined as proteins with more than one function that is distinct and independent of each other. The inactivation of one of them should not affect the second one and vice versa (Rodríguez-Saavedra et al., 2021).

Several studies have investigated the impact of HXK functions on the physiology of mammals and yeast (see review Rodríguez-Saavedra et al., 2021)**.** However, in plants, information is still lacking. It is speculated that they have a complex regulation of phytohormones and robust control of C and nitrogen sources, suggesting that moonlighting proteins in plants may be more abundant and interconnected with different signaling pathways (Rodríguez-Saavedra et al., 2021). It is also relevant to mention that HXKs have great potential to manipulate the productivity of plants eventually. Therefore, their study deserves more attention (Aguilera-Alvarado et al., 2019).

Physiologically, HXK can affect photosynthetic tissues and sink tissues. This occurs during all life cycle through the cross-talk between glucose signals (it's level) in hormones signaling pathways such as auxin, cytokinin, abscisic acid, gibberellic acid, brassinosteroid, and the growth regulator melatonin (Aguilera-

Alvarado; Sánchez-Nieto, 2017). Some studies showed that HXK could improve plant performance (Kelly et al., 2013, 2019). The increase in the expression of *AtHXK1* at guard cells in tomato (*Solanum lycopersicum*) caused a reduction in stomatal opening and a decrease in transpiration by approximately 20% without any adverse effects on growth (Kelly et al., 2013). Possibly, this occurred because glucose accumulated in leaves is carried by the transpiration stream toward the guard cells and is sensed by HXK stimulating stomatal closure. Additionally, this increase in the expression of *AtHXK1* under limited-water-supply conditions has exhibited drought avoidance and improved photosynthesis (Kelly et al., 2019), highlighting the potential of the HXK to improve the plant yield, which may be exploited in crop species.

Additional functions of HXKs in plants have been labeled mainly under salt stress. Apple (*Malus domesticus*) hexokinase 1, MdHXK1, seems to be involved in both regulations of anthocyanins biosynthesis and glucose-mediated salt stress tolerance (Hu et al., 2016; Sun et al., 2018). In high exogenous glucose levels, MdHXK1 interacts and phosphorylates an anthocyanin-associated bHLH transcription factor to stabilize it and thus enhance transcription of the anthocyanin biosynthesis genes (Hu et al., 2016). Furthermore, it was described that the same MdHXK1 contributes to glucose-mediated salinity tolerance by interacting and phosphorylating a protein vacuolar Na+/H+ exchanger, MdHNX1. The phosphorylation increased the stability of MdHNX1 and enhanced its Na+/H+ transport activity when *MdHXK1* was overexpressed (Sun et al., 2018). Other examples that HXK can help in plant productivity are related to pathogens. Overexpression of *AtHXK1* and *AtHXK2* in *Nicotiana benthamiana* improved the production of H_2O_2 that flowed to induce the expression of Pathogenesis-related Protein 1 (PR1) genes, resulting in plant resistance to the pathogen (Sarowar et al., 2008). In rice, the phosphorylation of a hexose called D-allose by HXKs induces ROS accumulation and high *PR* gene expression, increasing plant resistance to bacterial infection (Kano et al., 2013).

The target of Rapamycin multiprotein complex 1 (TORC1)

All living organisms need mechanisms to detect nutrients and favorable conditions that allow them to grow and develop. TORC1 plays this role and is a general nutrient sensor that coordinates the metabolism of sugars (Xiong et al.,

2013), nitrogen (Mubeen et al., 2018; Cao et al., 2019), and sulfur (Dong et al., 2017; Forzani; Turqueto Duarte; Meyer, 2018) during plant growth and development.

In yeast and mammals, TOR assembles into two multiproteic complex, TORC1 and TORC2 (Dobrenel et al., 2016; González; Hall, 2017). TORC1 is one of the most central and conserved regulatory pathways among sugar sensing mechanisms (Wullschleger; Loewith; Hall, 2006). The TORC2 function is related to cellular organization regulating actin cytoskeletal organization (Riggi et al., 2019). TOR itself forms TORC1, the lethal with sec thirteen protein 8 (LST8) and the Regulatory Associated Protein of TOR (RAPTOR) (Fig. 1a) (Dobrenel et al., 2016; González; Hall, 2017). On the other hand, TORC2 is composed of TOR, LST8, Rapamycin Insensitive Companion of TOR/AVO3" (RICTOR), and SAPK-interacting protein 1/AVO1" (SIN1) (Fig. 1b) (Dos D. Sarbassov et al., 2004; Gaubitz et al., 2016)

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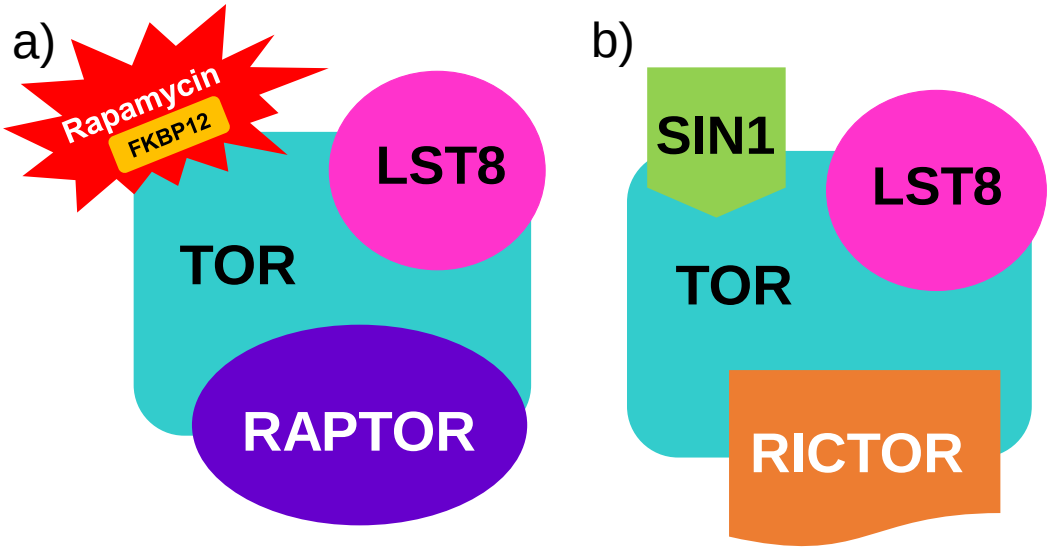

Figure 1: Representations of the TORC1 with drug rapamycin and protein FKBP12 (a) and TORC2 (b).

In plants, only orthologs of the TORC1 were identified so far. According to Serfontein and collaborators (2010), rather than being lost during evolution, RICTOR was acquired after the divergence between red algae, land plants, and other eukaryotes. Other components of the TORC2 may have also developed

later, during the evolution of animals and fungi. Thus, it is evident that the two complexes have separate evolutionary contexts (Van Dam et al., 2011).

The phosphorylation activity of TORC1 in yeast and mammals is blocked by rapamycin, an antibiotic produced by the bacterium *Streptomyces hygroscopicus*, causing interruption of cell division and growth inhibition (Dobrenel et al., 2016). This inhibitory activity is caused by the formation of a ternary complex of the rapamycin with the FKBP-rapamycin-binding domain (FRB) of TOR and a second binding protein, FK506-binding protein 12 (FKBP12) (Fig. 1a) (Xiong; Sheen, 2012). This inhibition in yeasts and mammals cells affects several conserved processes such as cell proliferation, protein translation, cell cycle, and embryogenesis (Ingargiola et al., 2020). TORC2 is insensitive to rapamycin and regulates cell survival, glucose metabolism, turgor pressure, and cell migration (Jacinto et al., 2004; Gaubitz et al., 2015; Riggi et al., 2019).

Although the presence of TORC1 in land plants is well documented, the elucidation of its functions lags behind other eukaryotes because only extremely high concentrations of rapamycin (Menand et al., 2002) or heterologous overexpression of yeast or human *FKBP12* can inhibit *A. thaliana* growth (Mahfouz et al., 2006; Sormani et al., 2007). The absence of reliable assays to monitor plant TORC1 activity further hampers the elucidation of its precise mode of action (Xiong; Sheen, 2012; Caldana et al., 2019). About the components of TORC1, TOR is a serine/threonine kinase that belongs to the class of phosphoinositol 3-kinase-related kinases (Wullschleger; Loewith; Hall, 2006). TOR knockout is embryo lethal, and transgenic manipulation of TOR expression levels positively correlates with growth (Deprost et al., 2007). In Arabidopsis and most species, TOR was identified as a single protein; however, more copies were verified only in three polyploidy plants (*Glycine max, Populus trichocarpa, and Brassica rapa*), indicating an intense selection pressure during plant evolution (Jamsheer K; Jindal; Laxmi, 2019). The subunits LST8 and RAPTOR are essential for TORC1 activation (Dobrenel et al., 2016).

Genetic manipulation of TORC1 components or their inhibition revealed a broad range of changes in genes and metabolites from primary and secondary metabolism (Moreau et al., 2012; Caldana et al., 2013; Dong et al., 2015; Salem et al., 2018). Suppression of *RAPTOR2* in rice or treatment with inhibitors like Torin2 or AZD8055 that target the ATP binding pocket of the TOR kinase domain

showed structurally altered thylakoids and photosynthesis ability (Sun et al., 2016). Interestingly, in C4 species like maize, TOR seems more sensitive to rapamycin inhibition, possibly caused by the conservation of its FKBP12 protein (Agredano-Moreno et al., 2007; Dinkova et al., 2007; Sotelo et al., 2010). On the other hand, seedlings of *Setaria viridis*, a suitable model for biomass crops, when submitted to treatment with AZD8055, showed a small magnitude of typical changes such as nutrient, amino acids, and growth-promoting partition than in Arabidopsis (Da Silva et al., 2021).

TORC1 promotes starch degradation through the induction of accumulation of β-Amylase1 (BAM1), responsible for starch degradation in guard cells. Sensing cellular energy status and nutrient availability is a crucial function of TORC1. For its activation, distinct signals (e.g., sugars and hormones) are required, and TORC regulates different primary, secondary, storage, and structural biomolecules. Still, the exact mechanisms remain to be elucidated (Caldana et al., 2019). Concerning sugars, glucose is necessary for TOR meristem activation during organogenesis in both root and shoot meristems, but the latter also needs light inputs evidencing the TORC role in connecting environmental cues and nutrients (Xiong et al., 2013; Li et al., 2017). How glucose activates the complex signaling is not yet understood, but it has been well-known that glucose–TORC1 signaling stimulates genes related to the biosynthesis of nucleotides, amino acids, proteins, and lipids while repressing genes involved in the catabolism of these products (Wu et al., 2019). Furthermore, it also induces stomatal opening in *Arabidopsis thaliana*. This regulation allows the availability of C to regulate starch metabolism and stomatal movement, ensuring optimal plant photosynthesis efficiency (Han et al., 2021). There is also a strong link between TORC1 and plant hormone signaling networks (Salem et al., 2018; Schepetilnikov et al., 2013; Song et al., 2017).

Physiologically, TORC1 is a crucial regulator in photosynthesis. Many studies with TOR dysfunctions/inhibition showed severe chloroplast defects and a broad regulation of plant photosynthesis-related genes (for more details, see Song et al., 2021). TORC1 also regulates leaf development, which was perceived through suppression of TOR by the inhibitor AZD8055 in Arabidopsis. The results showed that the cotyledon greening and expansion were eliminated and altered

the expression profile of photosynthesis-associated genes involved in chlorophyll biosynthesis, light reactions, and CO2 fixation (Dong et al., 2015).

In photosynthetic organisms, the inhibition of TOR provokes an immediate increase in free amino acids such as leucine, isoleucine, valine, tyrosine, tryptophan, lysine, β-alanine, histidine, proline, and gamma-aminobutyric acid (GABA). Recent experiments with the green algae *Chlamydomonas reinhardtii* revealed that this notable increase in amino acid levels was accompanied by increased N uptake and higher activity of the main enzymes involved in N assimilation (Mubeen et al., 2018). Furthermore, *de novo* amino acid synthesis continued to occur, a counterintuitive mechanism, as inactivation of TOR suppresses translation and mimics energy deprivation. It is believed that behind this regulatory process, there is a simple positive feedforward loop to reactivate TORC, like amino acids, and is essential building blocks for proteins, act as sources of energy, and C for various metabolic pathways (Mubeen et al., 2018).

In animal cells, there is evidence that the TORC1 is activated by free amino acids (Efeyan et al., 2013), which promotes protein synthesis and growth, while the lack of amino acids inhibits TOR activity. However, O'Leary and collaborators (2020) demonstrated that in leaf discs of *A. thaliana*, the TOR enzymatic activity is also influenced by amino acid levels. However, the mechanisms involved in this process have not yet been elucidated. Others metabolite activators of TORC1 also were discovered recently, such as spermidine, one major type of polyamines, and a nitrogen sink and signaling molecule that plays pivotal roles in eukaryotic cell growth (Salazar-Díaz et al., 2021). In this work, the authors exposed that spermidine stimulates the growth of maize and Arabidopsis seedlings through TOR signaling and provides its potential application for crop protection.

Sucrose-non-fermenting related protein kinase 1 (SnRK1)

In addition to TORC1, SnRK1, with largely opposed functions, also senses nutrients and responses to stress and growth, essential roles for plant survival (Soto-Burgos; Bassham, 2017). SnRK1 is activated under stressful and low sugar conditions and promotes energy-saving strategies by repressing anabolic processes that consume energy and growth (Margalha; Confraria; Baena-González, 2019). Part of this response has been projected to be exerted

by basic leucine zipper (bZIP) transcription factors (TFs) of group S1 as *bZIP11* (At4g34590) (Baena-González et al., 2007; Weiste et al., 2017)

SnRK1 kinases belong to the calcium-independent serine/threonine protein kinases family and are highly conserved in plants. Its functional homolog in animals and yeasts are AMP-dependent protein kinase (AMPK) and sucrose non-fermenting 1 (SNF1), respectively (Mckibbin et al., 2006). They are heterotrimeric complexes that consist of an α catalytic subunit (known as AKIN10 and AKIN11 in *A. thaliana*) and β and βγ regulatory subunits, which are necessary for stability, substrate specificity, location, and activity (Fig. 2) (Polge; Thomas, 2007). Plants also have *SnRK2* and *SnRK3* multigene families, intimately connected to ABA and abiotic stress signaling (Kulik et al., 2011). Transgenic plants with altered SnRK1 activity show several phenotypes like altered glucose sensitivity, accelerated senescence, and late-flowering (Williams et al., 2014), demonstrating that *SnRK2* and *SnRK3* cannot substitute *SnRK1*.

In plants, SnRK1 has multiple targets, directly regulating the activity of several enzymes and acting through gene expression regulation (Wurzinger et al., 2018). It also serves as the primary regulator of adaptive responses during energy deficiency, such as sugar deprivation, hypoxia, and other processes that consume a lot of energy like cell division (Baena-González et al., 2007; Dobrenel et al., 2016). Rice, maize, and *A. thaliana* have two genes encoding for SnRK1, each containing two domains, an N-terminal serine/threonine kinase domain that catalyzes the transfer of phosphate from nucleotide triphosphates (ATP) and a ubiquitin-associated (UBA) domain that contributes to the preservation of its catalytic activity (Emanuelle et al., 2018).

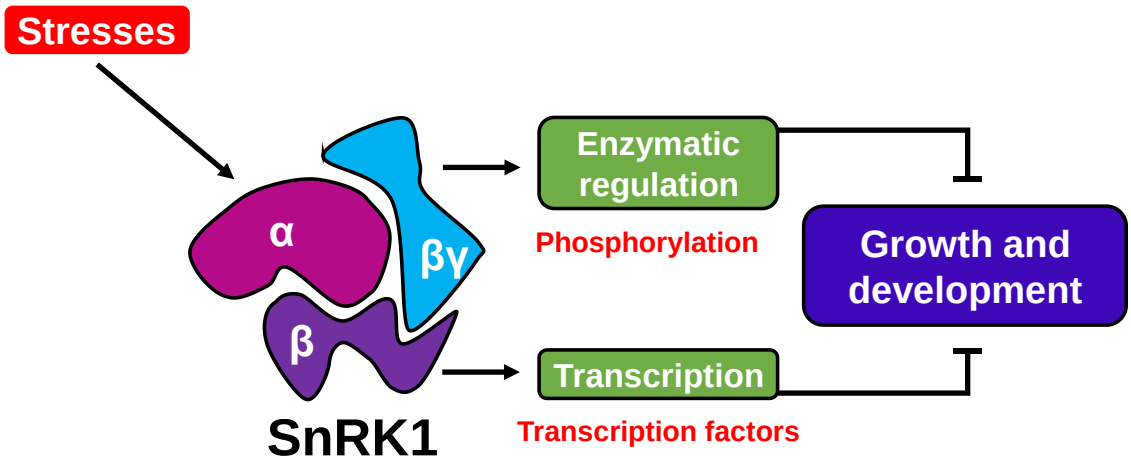

Figure 2. Simplified mechanism of action of the SnRK1 heterotrimeric complex. The SnRK1 complex is formed by a catalytic subunit α that plays a kinase role, and two regulatory subunits, β, and βγ. SnRK1 responds to stress scenarios and nutritional deprivation, directing responses, both via enzymatic regulation and from transcription control, to inhibit plant growth and development.

Sugars directly inhibit SnRK1. However, it is not entirely clear how SnRK1 responds to the availability of carbohydrates in plants. It appears that genes involved in several biosynthetic processes are repressed via SnRK1 when the cells have low amounts of sugars or energy and also mediate the induction of many genes related to nutrient remobilization processes (e.g., autophagy) as well as genes involved in general stress signaling (Emanuelle et al., 2018; Nukarinen et al., 2016). Some studies have shown SnRK1 acting in the regulation of starch biosynthesis in storage organs, such as seeds and tubers (Mckibbin et al., 2006).

The expression of sucrose synthase (SUSy) and ADP-glucose pyrophosphorylase (AGPase) in potatoes increased in response to the over-expression of *SnRK1*. The resulting phenotype led to tubers containing more starch and fewer soluble sugars (Mckibbin et al., 2006). In protoplasts of *A. thaliana,* many genes involved in primary and secondary metabolism have been identified as putative SnRK1 targets, which could consequently affect plant development (Baena-González et al., 2007; Baena-González; Sheen, 2008). Indeed, transgenic plants with altered *SnRK1* expression showed reduced growth, delayed flowering, and the onset of senescence (Baena-González et al., 2007). Interestingly, although transgenic rice overexpressing *OsSnRK1a* presented similar phenotypes, this kinase is proposed to be also involved in plant basal immunity against pathogen attack and mediates metabolic regulation of plant biotic relations (Filipe et al., 2018).

To overcome a critical energy limitation, plants limit the synthesis of protein and amino acids and nitrogen metabolism. An example is that in pea when *SnRK1* is repressed in embryos, there are lower levels of most of the organic and amino acids such as homoserine, threonine, glutamine, asparagine, glycine, valine, and arginine. In this same experiment, *SnRK1* represses higher levels of polyamines such as spermidine and putrescine, which are associated with stress responses. In general, this demonstrates that *SnRK1* repression inhibits the convention of C skeletons for amino acid synthesis because the levels

of sugars were higher or unaffected indicating reduced C partitioning into subsequent pathways (Radchuk et al., 2010).

SnRK1 phosphorylates and inactivates several enzymes or transcription factors that regulate catabolic processes as sucrose phosphate synthase (SPS), an enzyme involved in sucrose biosynthesis (Polge et al., 2008). Besides that, it also phosphorylates nitrate reductase (NR), an enzyme that assimilates nitrogen used for amino acid biosynthesis. This interaction points to two critical roles in plant C regulation through SnRK1: 1) potentially regulates SPS activity. Therefore, SnRK1 becomes a key regulator for source C-flux and acclimating to C supply. For example, increased SPS activity in a leaf could result in more remarkable sucrose synthesis, and more sucrose would be available for export to source tissues; and 2) the potential phosphorylation of SnRK1 demonstrates that SnRK1 can regulate nitrogen assimilation and thereby amino acid biosynthesis and this is particularly important because it connects C signaling and nitrogen (Halford et al., 2004; Halford, 2005).

Trehalose-6-phosphate (T6P)

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is a nonreducing disaccharide consisting of two glucose units present in bacteria, archaea, fungi, invertebrates, and plants (Fichtner et al., 2021). Unlike the other sugar sensors mentioned above, the metabolite T6P is the intermediate of the trehalose pathway. It plays various functions as transport sugar, osmolyte, stress protectant, and reserve (Figueroa; Lunn, 2016). Two consecutive steps catalyze its biosynthesis in plants: (1) the transfer of glucose from UDP-glucose to Glc6P producing T6P and uridine diphosphate (UDP) catalyzed by the enzyme trehalose-6-phosphate synthase (TPS); and subsequently (2) the dephosphorylation of T6P to form trehalose and inorganic phosphate catalyzed by the enzyme trehalose phosphate phosphatase (TPP) (Cabib; Leloir, 1958). Trehalose is cleaved by trehalase (TRE) into two glucose (Glc) molecules (Fig. 3). Multigene families encode TPS and TPP while TRE is by a single gene.

Most TPS isoforms have a regulatory rather than a catalytic function, and their expression responds quickly to sugar levels and the diurnal cycle (Bläsing et al., 2005). In contrast, all TPP isoforms are catalytic, and their expression

reacts strongly to environmental changes and abiotic stresses such as cold, drought, hypoxia, and nitrate availability (Yang et al., 2012; Henry et al., 2014).

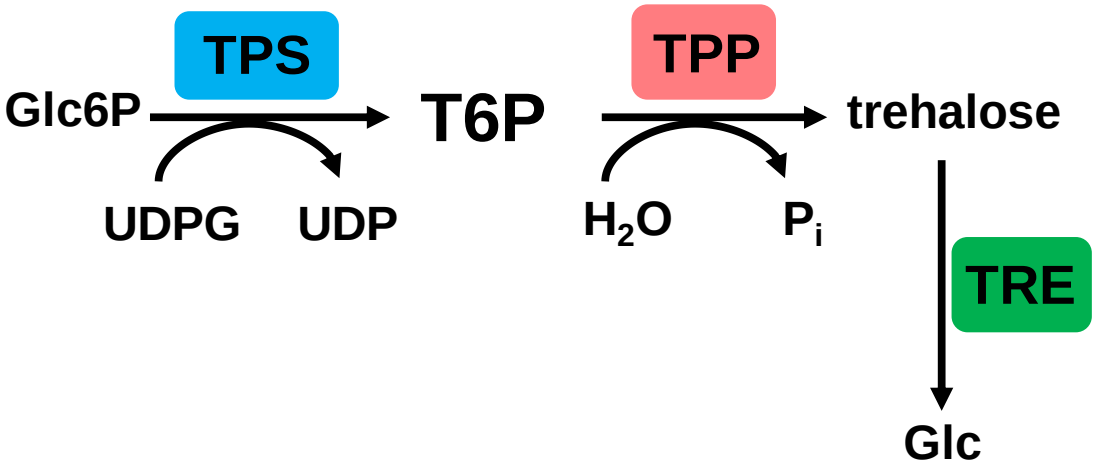

Figure 3. Trehalose metabolism in plants.

Trehalose levels are generally low to contribute to the reserve or transport of sugar, and these functions are already performed by sucrose (Lunn et al., 2014). However, trehalose metabolism is essential in plants, with the loss of TPS catalytic activity being lethal to the embryo and severely affecting plant growth and development (Eastmond et al., 2002). The loss of specific TPP isoforms also affects plant morphology and the constitutive overexpression of *TPS* or *TPP* (Schluepmann et al., 2003). Transgenic *A. thaliana* plants constitutively overexpressing *Escherichia coli* TPS (*otsA*) or TPP (*otsB*) show antagonistic phenotypic effects (Schluepmann et al., 2003). The plants had small leaves, highly branched inflorescences, and flowered early for TPS. For TPP, the plants had large leaves, few inflorescence branches, and late flowering (Schluepmann et al., 2003). Together, the opposite phenotypes of modulation of TPS and TPP activity in transgenic plants confirm that such alterations are due to changes in T6P levels rather than trehalose itself (Schluepmann et al., 2003).

T6P is a specific sucrose sensor (Lunn et al., 2014; Yadav et al., 2014) that regulates plant metabolism, growth, and development. Starch degradation at night is also affected by T6P. It is part of the mechanism to maintain sucrose levels at an acceptable range for being used as a supply for cellular growth and metabolic activities (Martins et al., 2013; Yadav et al., 2014). Rice plants overexpressing *OsTPS1* have increased trehalose levels and improved their

tolerance to cold, salinity, and drought stresses (Li et al., 2011). These same authors overexpressed other TPS (*OsTPS2*, *OsTPS4*, *OsTPS5*, *OsTPS8*, and *OsTPS9*), which also influenced stress tolerance in this species. In maize, the classical mutant *RAMOSA3* is defective in a TPP isoform that alters the branching pattern in male and female inflorescences (Gallavotti et al., 2010).

Integration of sugars sensing pathways

Although briefly described separately, the mentioned pathways can interact directly or indirectly. In yeast, T6P controls glycolysis by inhibiting HXK *in vitro* (Blázquez et al., 1993). On the other hand, no experimental evidence for inhibition of HXK activity by T6P has so far been reported (Eastmond et al., 2002). However, as mentioned in the section on HXK in plants, few works still characterize all members of this family. Therefore, it is impossible to exclude the possibility that plants contain some isoforms of HXK that are sensitive to T6P (Claeyssen; Rivoal, 2007).

Interestingly, some interactions between T6P and SnRK1 have been demonstrated. SnRK1 directly phosphorylates some TPS isoforms, and T6P levels can inhibit its activity in developing tissues. Therefore, SnRK1 is both a target of T6P and a regulator of its quantity in plant cells (Yadav et al., 2014). SnRK1 manipulation in *A. thaliana* alters the sucrose and T6P relationship, influencing how the sucrose content is translated into T6P accumulation and modulating the flux of C to the tricarboxylic acid cycle. This reveals that under favorable growth conditions, SnRK1 plays a role in sucrose homeostasis, and its activity is influenced by diel fluctuations in T6P levels (Peixoto et al., 2021). These findings expose meaningful interactions between sugar pathways and sensors and increase the complexity of the network of regulatory mechanisms that coordinate plant growth and metabolism.

SnRK1 is crucial for seed germination and seedling growth in rice (Lu et al., 2007; Lin et al., 2014). It was demonstrated that maize plants at the silking stage subjected to salt stress present mild photosynthesis reduction and increased sugar concentrations (sucrose, glucose, and T6P) in their leaves. In contrast, reproductive tissues had the expression of several SnRK1 targets severely affected (Henry et al., 2015). These authors confirmed that T6P could inhibit the *in vitro* activity of SnRK1 in maize leaves also under salt stress. Kernel

excision from the plant reduced T6P content consistent with the activation of SnRK1 based on the resulting changes in transcript abundance (Bledsoe et al., 2017).

Extreme events such as very high or low temperatures, drought, overflowing, or attacks from various pathogens are major yield-limiting factors, reducing crop productivity by >50% (FAO, 2021). To deal with this, plants trigger responses that range from rapid protective apparatuses to developmental modifications, finally promoting stress tolerance and survival at the expense of growth. In plants, one central nutrient-sensing kinase with increasing connections to stress responses and growth is the TORC1-SnRK1 modulation (Margalha; Confraria; Baena-González, 2019).

In *in vitro* assays, SnRK1 interacts with TORC1. It inhibits its activity by phosphorylation of the RAPTOR protein, a trade-off that contributes to C partition in the plant through molecular mechanisms not fully understood (Nukarinen et al., 2016). Studies suggest that SnRK1 more directly limits growth and can stimulate cell death mediated by autophagy (possibly inhibiting TORC1) (Baena-González et al., 2007; Baena-González; Sheen, 2008; Baena-González; Hanson, 2017).

Assuming that an abundance of glucose is also perceived as a high nutrient and energy state, in animals, HXK activity not only affects the sequence of glycolysis reactions but also can interfere in the signaling pathway of the TORC1 through changing the levels of Glc6P (Roberts et al., 2014), indicating a connection between TORC1 with the catalytic activity of HXKs. However, advanced studies are still needed to elucidate this connection.

A schematic image with an integration of all these pathways is illustrated in Fig. 4.

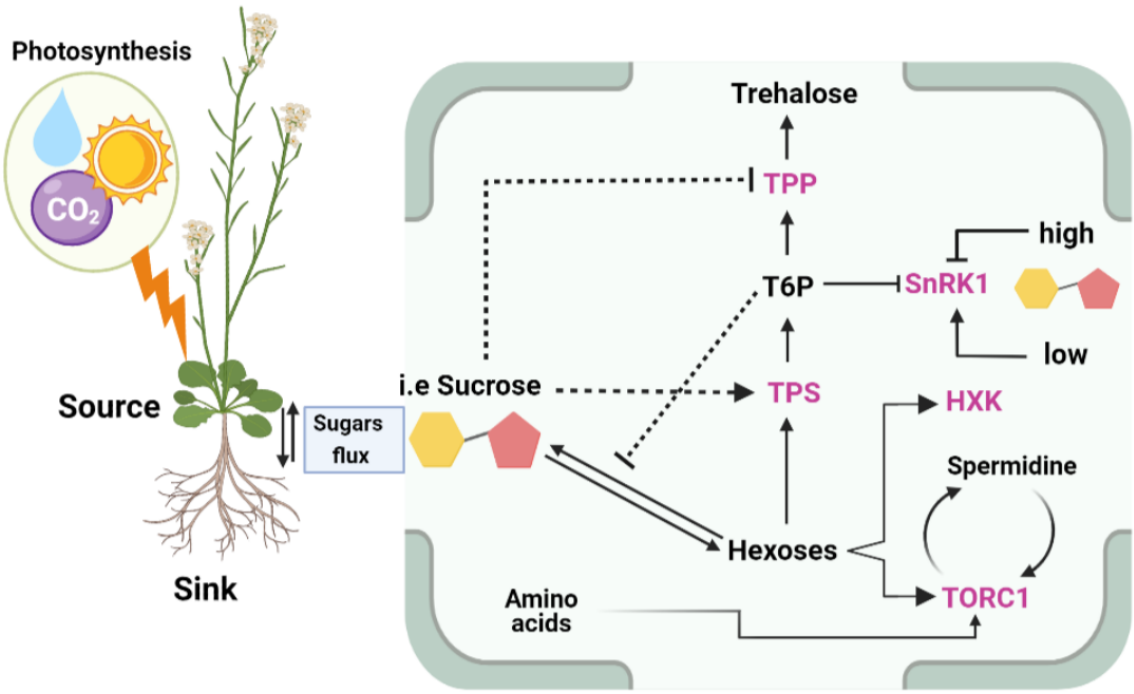

Figure 4. Integration of sugar sensing and signaling pathways. Photosynthesis occurs in leaves (source tissues) that produce sucrose, which is transported to sink tissues. Increased sucrose leads to a respective rise in T6P levels through decreased TPP activity or increased TPS activity. T6P regulates the partitioning of hexoses acting as signal and negative feedback regulators of sucrose levels. T6P regulates the consumption of sucrose mediated in part by inhibition of SnRK1, which is activated by the low energy status of sucrose. Any changes in hexose levels are likely to trigger other sugar signaling responses mediated by TORC1 and HXK. TORC1 is activated by amino acids and also senses and regulates spermidine metabolism. Black lines with and without arrows indicate activation and inhibition, respectively. Dashed black lines represent hypothetical interactions and fade lines mean that the mechanism is not yet fully elucidated (amino acids) and that there are other intermediate components in the process (spermidine). The pink letters represent the enzymes and the black ones the metabolites. Figure based on (Figueroa; Lunn, 2016; O'Leary et al., 2020; Salazar-Díaz et al., 2021).

Sugar sensing in sugarcane

Sugarcane is one of the essential crops known as a feedstock for sugar and bioethanol production, and Brazil stands out worldwide for its production (Cursi et al., 2022). Most bioethanol production depends on the first generation, which consists of extracting and concentrating sucrose to be fermented (see review De Souza et al., 2014). Sugarcane accumulates extremely high sucrose concentrations (up to 600 mM) in its culm (Zhu; Komor; Moore, 1997), and this complex characteristic has been studied quite extensively, mainly concerning sucrose synthesis, transport, and storage (Hu et al., 2018; Shi et al., 2019; Ma et al., 2020; Khan et al., 2021; Qin et al., 2021).

Sucrose metabolism occurs in the cytosol and involves the action of several enzymes (Wang et al., 2013). Similar to trehalose, sucrose synthesis is

also catalyzed in two steps. First, the sucrose phosphate synthase (SPS) transfers the glucosyl from UDP-glucose to fructose-6-phosphate (Fru6P), producing sucrose-6-phosphate (Suc6P) and UDP. Then, the enzyme sucrose-phosphatase (SPP) removes the phosphate group from Suc6P to form sucrose. Sucrose synthase (SUSy) catalyzes the reversible conversion of UDP-glucose and fructose into sucrose and UDP (Lunn; Macrae, 2003). After being synthesized in the mesophyll cells of source leaves, sucrose needs to be transported via symplastic or apoplastic through specific transporters, such as Sucrose Transporters (SUTs) and Sugars Will Eventually Be Exported Transporter (SWEET). This C source is used for growth and development in sink organs. When the photosynthetic capacity of the source exceeds the demand of sink tissues, the excess of photoassimilates is stored (Mccormick; Cramer; Watt, 2006)**.**

This accumulation occurs in parenchyma cells in the stems of sugarcane and increases with internode maturity. Sucrose accumulation can result in adjustments of the rates of sucrose phloem loading due to reductions in sink strength, consequently affecting the source-sink relationship (Mccormick; Cramer; Watt, 2006). During the day-night cycle and different phases of sugarcane development, several anabolic processes occur, contributing to variations in the accumulation and use of sugars. This relationship among synthesis, use, and storage is influenced by environmental changes and dynamics of carbohydrates controlled and interconnected within the plant development (De Souza et al., 2018).

In sugarcane, the components of the sucrose pathway mentioned above are potential targets for improving varieties, aiming at more significant sucrose accumulation. Some of these enzymes are used as markers. For instance, in young internodes, SUSy contributes to sucrose synthesis (Botha; Black, 2000), and SPS in leaves has a good correlation between its activity and sucrose content (Grof et al., 2007). Although transgenic lines overexpressing *SPS* have increased sucrose content, they showed higher soluble acid invertase activity, a sucrose degrading enzyme, consistent with the elevated hexose levels that surpass the magnitude of sucrose increment in leaves when compared to control plants (Anur et al., 2020). These results indicate that such manipulation will not necessarily allow an increase in yield.

Another example is the transgenic overproduction of trehalulose, a sucrose isomer that is naturally present in various sucrose-containing foods. Trehalulose concentration in juice increased with internode maturity, but plants have thinner stems due to weaker initial growth (Hamerli; Birch, 2011). This characteristic can affect the time of field propagation to obtain stalks of average commercial size and consequently increase its cost (Hamerli; Birch, 2011). Possibly, these transgenic plants are not very successful because (i) the targeted proteins exert little control in the pathway (Stitt; Lunn; Usadel, 2010), (ii) the existence of compensatory mechanisms, like post-translational changes, and (iii) the regulation of other isoforms or different enzymes. Besides, none of these transgenic sugarcane manipulations considered the importance of sugar sensors and signals.

In other species, all sugar sensors mentioned in the previous sessions (HXK, TORC1, SnRK1, and T6P) regulate essential functions associated with cell growth, development, protein synthesis, and cellular metabolism. In sugarcane, despite efforts, there are few studies with sugars sensors and signals in which critical knowledge can potentially ensure positive implications in plant performance and crop yields. So far, it is known that three copies of HXK were identified and enzymatically characterized (Hoepfner; Botha, 2004). More recently, an *ShHXK8* was highly expressed in mature leaves and young internodes (Wang et al., 2019). The presence of seven haplotypes of TOR is described in the cultivar R570 (Vilela et al., 2017). Notoriously, more information on SnRK is available: twenty-two expressed sequence tags (ESTs) were identified. However, only three were SnRK1 (Carraro; Lambais; Carrer, 2001). Other studies also identify two SnRK1 sequences (*ScSnRK1* and *ScSnRK1*-2), which were differentially expressed between high and low brix cultivars, being *ScSnRK1*-2 induced by sucrose (Papini-TerzI et al., 2009; Ferreira et al., 2016). An *SnRK1* transcript was also up-regulated in cultivars' leaves with low sugar content (De Maria Felix et al., 2009). Besides, SnRK2 and SnRK3 were also found and characterized (Priji; Hemaprabha, 2015; Li et al., 2017).

Concerning the T6P pathway, orthologous isoforms of AtTPS1 Class I (*STPS1*) and AtTPS7 Class II (*STPS2*) were found in cultivars tolerant and susceptible to water stress. *STPS1* was up-regulated in the tolerant cultivar, and *STPS2* had no significant changes in any of the evaluated cultivars (Junior

et al., 2013). These results raised the possibility that increased *STPS1* expression correlates with the osmoprotectant function of the T6P pathway as it does in rice (Li et al., 2011). More recently, other studies identified TPS in *Saccharum* spp. hybrids, which exhibit divergent expression in response to simulated drought, salinity, and ABA stresses. Since *ScTPS* genes function in sugarcane adaptation to environmental stimuli, they might be used as molecular markers for increased stress resistance (Hu et al., 2020).

TPS and TPP transcripts were found up and down-regulated, respectively, during cold-girdling manipulation of the source and sink relationship (Mccormick; Cramer; Watt, 2008). This condition represses the expression of genes related to photosynthesis and increases the expression of genes associated with better photoassimilate partitioning (Mccormick; Cramer; Watt, 2008). Transgenic sugarcane lines overexpressing *TPS* and *TPP* showed increased trehalase activity and correspondent trehalose biosynthesis but did not have an increment in sugars like sucrose and glucose (O'neill et al., 2012). Otherwise, the independent overexpression of the *Escherichia coli otsA* (TPS) and *otsB* (TPP) genes in sugarcane had a reduction and increase in sucrose levels in the respectively transgenic plants (Gabriel et al., 2021).

These results reinforce that sugar sensing mechanisms are complex and have not been much explored in sugarcane. However, they are highly relevant to increasing crop yield, which depends on a better understanding of how plants coordinate their metabolism and responses to diverse environmental conditions during their life cycle. Field experiments are crucial to assess how plants respond to natural variations in this context.

Thesis contextualization

The studies conducted by the Laboratory of Ecological Plant Physiology (LAFIECO), headquarter of the National Institute of Science and Technology of Bioethanol (INCT / Bioethanol), investigates, through systemic analyzes covering the physiology, biochemistry, and molecular biology, the production of bioenergy from plant biomass as a strategy to reduce the impacts of global climate change.

Sugarcane has an impressive biomass accumulation (Singh et al., 2018; Figueiredo et al., 2020) and is an appropriate system because approximately one-third of the total sugarcane energy potential is present in the sugar fraction

in the culm, mainly in the form of sucrose. To comprehend its growth and development is vital to understanding points of improvement and increasing productivity. In addition to that, the crucial role of sugar sensing in plant growth can be a turning point in physiological and morphological development programs knowledge and a biological C-flux control tool for biomass accumulation. Besides, HXK, TORC1, SnRK1, and T6P play multifaceted roles in plant response to different kinds of abiotic stresses, functioning as either positive or negative regulators depending on the type and the spatio-temporal mechanism of stress (Fu; Wang; Xiong, 2020). However, it is essential to point out that the amount of sucrose that sugarcane accumulates is different from other species. Here, we report studies that validate the information about its sugar sensing mechanisms. In this context, our group focused on investigating molecular and biochemical aspects of the sugar sensing and signaling responses mechanisms in sugarcane, seeking a future crop application to enhance its yields, apart from breeding strategy, through a deep comprehension of biomass accumulation.

To understand the dynamics of production and accumulation of carbohydrates throughout the development of sugarcane, we used data and materials obtained from a field experiment previously conducted by our group during 12 months of growth and development before sugarcane harvest (De Souza et al., 2018). This work analyzed the dynamics of leaf gas exchange and the accumulation of carbohydrates in leaves, stems, and roots during the diurnal cycle (De Souza et al., 2018). It has been observed that the daily rhythm influences the metabolism of sugars throughout the growing cycle. A crucial physiological transition occurred between three and six months of age, leading to changes in carbohydrate metabolism. After six months, the plants started to store a large amount of sucrose in their culm that continued until 12 months, when it reached the maximum concentration. Photosynthesis on leaf +1 decreased during development so that, if flowering were induced, most of the stored sugars would be consumed. Harvest takes place earlier to obtain sugars which are taken to fermentation and transformed into bioethanol.

Thus, a rapid growing season (up to three months) occurs before this physiological transition, where the plants establish themselves in the prevailing environmental conditions. After this period, when a physiological balance between the organs is reached, storage-related processes are activated. Several

mechanisms involved in the control and signaling of source and sink tissues are necessary for this transition. It remains to be investigated how the sugar sensing pathways orchestrate metabolic and external signals among different tissues and organs along with the sugarcane development.

In this scenario, it is critical to use the same samples from the field experiment to identify the sugar sensor genes that modulate C-flux. Since carbohydrates have been previously studied, quantifying various metabolites such as amino acids and polyamines is equally essential. In addition to controlling C and amino acid metabolism, the signaling pathways must cross-talk since these metabolites are based on C skeletons. In addition, the samples will be used for more precise analyses of gene expression profiles. These analyses aim to improve sugarcane, both for its adaptation to climate change and to increase its yield by identifying marker genes. It is essential to highlight that gene identification in this species is a significant challenge, as the sugarcane genome is highly complex, interspecific, polyploidy, and aneuploidy (Thirugnanasambandam; Hoang; Henry, 2018).

Thus, in this thesis, several sugar sensors and signaling pathways have been identified and characterized by analyses of gene structure, phylogeny, functional domains of proteins, and *in silico* expression levels of HXK, TORC1, SnRK1, and enzymes from trehalose metabolism. For that, protein targets from model plant species were used as queries to identify the groups of orthologous genes (OGs) they belonged to, within Viridiplantae, in the database EggNOG (Huerta-Cepas et al., 2016). The known OGs were then used to identify genes belonging to the same groups in species of the subfamily Panicoideae, whose genomes are publicly available, including also sugarcane, where a mixture of seven genomic and transcriptomic datasets was used. Post-translational modifications (*N*-glycosylation), three-dimensional structure modeling, and residues involved in catalysis and substrate binding were analyzed only regarding TPS sequences. This bioinformatics analysis paves the way to understand the physiological roles of sugar sensing and signaling pathways in sugarcane.

This thesis aimed at a systemic understanding of the flow of C in sugarcane, integrating physiology and biochemistry data from De Souza et al. (2018) and amino acid and polyamine profiles from sugarcane leaves and stalks as the enzymatic activity of the HXK. Shortly, we intend to incorporate the

expression of genes related to sugar metabolism into this data set. We expect this and the future analyses to provide a stronger foundation for developing strategies based on manipulating physiological and molecular characteristics proposed to improve crop performance further. The workflow for the development of this thesis is presented in Fig. 5.

The analyses related to the metabolism of amino acids and polyamines were carried out in collaboration with the Laboratory of Plant Cell Biology (BIOCEL) at IB-USP.

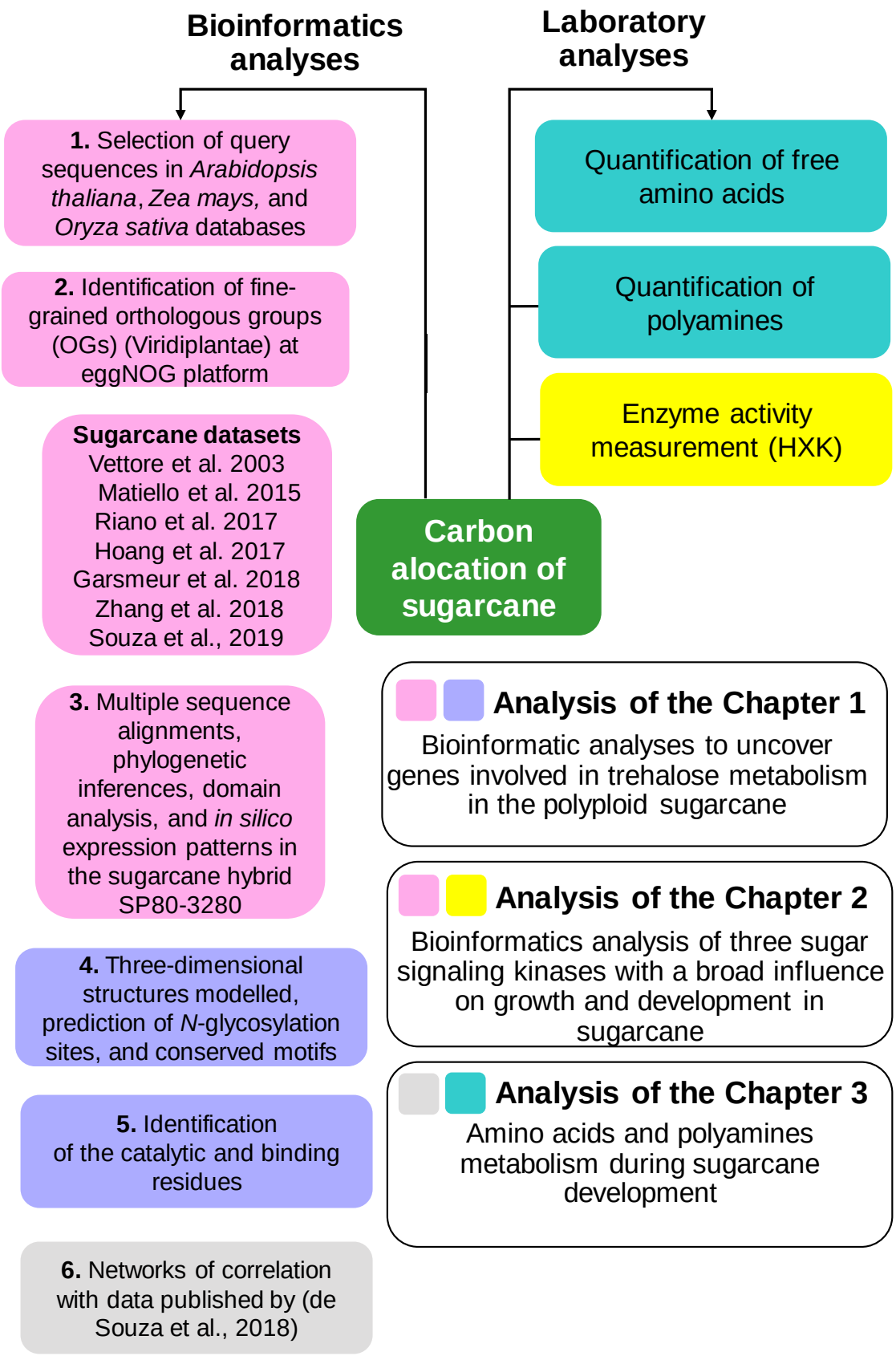

Figure 5. Thesis development workflow.

Reference

AGREDANO-MORENO, L. T. et al. Distinctive expression and functional regulation of the maize (*Zea mays* L.) TOR kinase ortholog. **Molecular bioSystems**, v. 3, n. 11, p. 794–802, nov. 2007.

AGUILERA-ALVARADO, G. P. et al. Biochemical properties and subcellular localization of six members of the HXK family in maize and its metabolic contribution to embryo germination. **BMC plant biology**, v. 19, n. 1, p. 27, 15 jan. 2019.

AGUILERA-ALVARADO, G. P.; SÁNCHEZ-NIETO, S. Plant Hexokinases are Multifaceted Proteins. **Plant & Cell Physiology**, v. 58, n. 7, p. 1151–1160, 1 jul. 2017.

ANUR, R. M. et al. Overexpression of sucrose phosphate synthase enhanced sucrose content and biomass production in transgenic sugarcane. **Plants**, v. 9, n. 2, p. 1–11, 2020.

BAENA-GONZÁLEZ, E. et al. A central integrator of transcription networks in plant stress and energy signaling. **Nature**, v. 448, n. 7156, p. 938–942, 2007.

BAENA-GONZÁLEZ, E.; HANSON, J. Shaping plant development through the SnRK1-TOR metabolic regulators. **Current Opinion in Plant Biology**, v. 35, p. 152–157, fev. 2017.

BAENA-GONZÁLEZ, E.; SHEEN, J. Convergent energy and stress signaling. **Trends in Plant Science**, v. 13, n. 9, p. 474–482, 2008.

BEARD, S. J. et al. Assessing climate change's contribution to global catastrophic risk. **Futures**, v. 127, p. 102673, 1 mar. 2021.

BLÄSING, O. E. et al. Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. **Plant Cell**, v. 17, n. 12, p. 3257–3281, 2005.

BLÁZQUEZ, M. et al. Trehalose-6-phosphate, a new regulator of yeast glycolysis that inhibits hexokinases. **FEBS letters**, 1993.

BLEDSOE, S. W. et al. The role of Tre6P and SnRK1 in maize early kernel development and events leading to stress-induced kernel abortion. **BMC Plant Biology**, v. 17, n. 1, p. 74, 12 abr. 2017.

BOTHA F, BLACK KG. Sucrose phosphate synthase and sucrose synthase activity during maturation of internodal tissue in sugarcane. Aust J Plant **Physiol.;**27:81–5, 2000.

BUCKERIDGE, M. S. et al. Ethanol from sugarcane in Brazil: a 'midway' strategy for increasing ethanol production while maximizing environmental benefits. **GCB Bioenergy**, v. 4, n. 2, p. 119–126, 2012.

BUCKERIDGE, M. S.; SANTOS, W. D. DOS; SOUZA, A. P. DE. Routes for cellulosic ethanol in brazil. In: CORTEZ, L. A. B. (Ed.). . **Sugarcane bioethanol — R&D for Productivity and Sustainability**. [s.l.] Editora Edgard Blücher, 2014. p. 365–380.

CABIB, E.; LELOIR, L. F. The biosynthesis of trehalose phosphate. **Journal of Biological Chemistry**, v. 231, n. 1, p. 259–275, mar. 1958.

CALDANA, C. et al. Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in *Arabidopsis thaliana*. **Plant Journal**, v. 73, n. 6, p. 897–909, 2013.

CALDANA, C. et al. The magic 'hammer' of TOR: the multiple faces of a single pathway in the metabolic regulation of plant growth and development. **Journal of Experimental Botany**, v. 70, n. 8, p. 2217–2225, 15 abr. 2019.

CALDERAN-RODRIGUES, M. J. et al. Applying Molecular Phenotyping Tools to Explore Sugarcane Carbon Potential. **Frontiers in Plant Science**, v. 12, 2021.

CAO, P. et al. Homeostasis of branched-chain amino acids is critical for the activity of TOR signaling in Arabidopsis. **eLife**, v. 8, p. e50747, 6 dez. 2019.

CARRARO, D. M.; LAMBAIS, M. R.; CARRER, H. In silico characterization and expression analyses of sugarcane putative sucrose non-fermenting-1 (SNF1) related kinases. **Genetics and Molecular Biology**, v. 24, p. 35–41, dez. 2001.

CLAEYSSEN, E.; RIVOAL, J. Isozymes of plant hexokinase: occurrence, properties and functions. **Phytochemistry**, v. 68, n. 6, p. 709–731, mar. 2007.

CURSI, D. E. et al. History and Current Status of Sugarcane Breeding, Germplasm Development and Molecular Genetics in Brazil. **Sugar Tech**, v. 24, n. 1, p. 112–133, fev. 2022.

DA SILVA, V. C. H. et al. Shedding Light on the Dynamic Role of the “Target of Rapamycin” Kinase in the Fast-Growing C4 Species *Setaria viridis*, a Suitable Model for Biomass Crops. **Frontiers in Plant Science**, v. 12, p. 637508, 2021.

DE MARIA FELIX, J. et al. Expression Profile of Signal Transduction Components in a Sugarcane Population Segregating for Sugar Content. **Tropical Plant Biology**, v. 2, n. 2, p. 98–109, 1 jun. 2009.

DE SOUZA, A. P. et al. Sugarcane as a Bioenergy Source: History, Performance, and Perspectives for Second-Generation Bioethanol. **BioEnergy Research**, v. 7, n. 1, p. 24–35, 1 mar. 2014.

DE SOUZA, A. P. et al. Diurnal variation in gas exchange and nonstructural carbohydrates throughout sugarcane development. **Functional Plant Biology**, v. 45, n. 8, p. 865–876, 2018.

DEPROST, D. et al. The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. **EMBO reports**, v. 8, n. 9, p. 864–870, set. 2007.

DINKOVA, T. D. et al. Dissecting the TOR–S6K signal transduction pathway in maize seedlings: relevance on cell growth regulation. **Physiologia Plantarum**, v. 130, n. 1, p. 1–10, 2007.

DOBRENEL, T. et al. TOR Signaling and Nutrient Sensing. **Annual Review of Plant Biology**, v. 67, p. 261–285, 29 abr. 2016.

DONG, P. et al. Expression profiling and functional analysis reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling pathways in Arabidopsis. **Frontiers in Plant Science**, v. 6, 2015.

DONG, Y. et al. Sulfur availability regulates plant growth via glucose-TOR signaling. **Nature Communications**, v. 8, n. 1, p. 1174, 27 out. 2017.

DOS D. SARBASSOV et al. Rictor, a Novel Binding Partner of mTOR, Defines a Rapamycin-Insensitive and Raptor-Independent Pathway that Regulates the Cytoskeleton. **Current Biology**, v. 14, n. 14, p. 1296–1302, 27 jul. 2004.

EASTMOND, P. J. et al. Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. **Plant Journal**, v. 29, n. 2, p. 225–235, 2002.

EFEYAN, A. et al. Regulation of mTORC1 by the Rag GTPases is necessary for neonatal autophagy and survival. **Nature**, v. 493, n. 7434, p. 679–683, 31 jan. 2013.

EMANUELLE, S. et al. The UBA domain of SnRK1 promotes activation and maintains catalytic activity. **Biochemical and Biophysical Research Communications**, v. 497, n. 1, p. 127–132, 2018.

FENG, J. et al. Biochemical and structural study of Arabidopsis hexokinase 1. **Acta Crystallographica. Section D, Biological Crystallography**, v. 71, n. Pt 2, p. 367–375, fev. 2015.

FERRARI, S. et al. Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. **Frontiers in Plant Science**, v. 4, 2013.

FERREIRA, S. S. et al. Co-expression network analysis reveals transcription factors associated to cell wall biosynthesis in sugarcane. **Plant Molecular Biology**, v. 91, p. 15–35, 2016.

FICHTNER, F. et al. Sugar and Nitrate Sensing: A Multi-Billion-Year Story. **Trends in Plant Science**, v. 26, n. 4, p. 352–374, abr. 2021.

FIGUEIREDO, R. et al. The sugarcane ShMYB78 transcription factor activates suberin biosynthesis in *Nicotiana benthamiana*. **Plant Molecular Biology**, v. 104, n. 4, p. 411–427, 1 nov. 2020.

FIGUEROA, C. M.; LUNN, J. E. A Tale of Two Sugars: Trehalose 6-Phosphate and Sucrose. **Plant physiology**, v. 172, n. 1, p. 7–27, set. 2016.

FILIPE, O. et al. The energy sensor OsSnRK1a confers broad-spectrum disease resistance in rice. **Scientific Reports**, v. 8, n. 1, p. 1–12, 2018.

FORZANI, C.; TURQUETO DUARTE, G.; MEYER, C. The Plant Target of Rapamycin Kinase: A connecTOR between Sulfur and Growth. **Trends in Plant Science**, v. 23, n. 6, p. 472–475, 2018.

FU, L.; WANG, P.; XIONG, Y. Target of Rapamycin Signaling in Plant Stress Responses. **Plant Physiology**, v. 182, n. 4, p. 1613–1623, 1 abr. 2020.

GABRIEL, C. et al. Genetic manipulation of trehalose-6-phosphate synthase results in changes in the soluble sugar profile in transgenic sugarcane stems. **Plant Direct**, v. 5, n. 11, p. e358, nov. 2021.

GALLAVOTTI, A. et al. The control of axillary meristem fate in the maize ramosa pathway. **Development (Cambridge, England)**, v. 137, n. 17, p. 2849–2856, 1 set. 2010.

GAUBITZ, C. et al. Molecular Basis of the Rapamycin Insensitivity of Target Of Rapamycin Complex 2. **Molecular Cell**, v. 58, n. 6, p. 977–988, 18 jun. 2015.

GAUBITZ, C. et al. TORC2 Structure and Function. **Trends in Biochemical Sciences**, v. 41, n. 6, p. 532–545, jun. 2016.

GONZÁLEZ, A.; HALL, M. N. Nutrient sensing and TOR signaling in yeast and mammals. **The EMBO journal**, v. 36, n. 4, p. 397–408, 15 fev. 2017.

GRANOT, D. et al. Substantial roles of hexokinase and fructokinase in the effects of sugars on plant physiology and development. **Journal of Experimental Botany**, v. 65, n. 3, p. 809–819, 1 mar. 2014.

GROF, C. P. L. et al. Sucrose-Phosphate Synthase, a Biochemical Marker of High Sucrose Accumulation in Sugarcane. **Crop Science**, v. 47, n. 4, p. 1530–1539, 2007.

HALFORD, N. G. et al. Highly conserved protein kinases involved in the regulation of carbon and amino acid metabolism. **Journal of Experimental Botany**, v. 55, n. 394, p. 35–42, jan. 2004.

HALFORD, N. G. Regulation of Carbon and Amino Acid Metabolism: Roles of Sucrose Nonfermenting-1-Related Protein Kinase-1 and General Control Nonderepressible-2-Related Protein Kinase. In: **Advances in Botanical Research**. Incorporating Advances in Plant Pathology. [s.l.] Academic Press, 2005. v. 43, p. 93–142.

HAMERLI, D.; BIRCH, R. G. Transgenic expression of trehalulose synthase results in high concentrations of the sucrose isomer trehalulose in mature stems of field-grown sugarcane. **Plant Biotechnology Journal**, v. 9, n. 1, p. 32–37, 2011.

HAN, C. et al. TOR promotes guard cell starch degradation by regulating the activity of β-AMYLASE1 in Arabidopsis. **The Plant Cell**, p.307, 17 dez. 2021.

HEIDRICH, F. et al. AMPK - Activated Protein Kinase and its Role in Energy Metabolism of the Heart. **Current Cardiology Reviews**, v. 6, n. 4, p. 337–342, nov. 2010.

HENRY, C. et al. The trehalose pathway in maize: conservation and gene regulation in response to the diurnal cycle and extended darkness. **Journal of experimental botany**, v. 65, n. 20, p. 5959–5973, nov. 2014.

HENRY, C. et al. Differential Role for Trehalose Metabolism in Salt-Stressed Maize. **Plant Physiology**, v. 169, n. 2, p. 1072–1089, out. 2015.

HOEPFNER, S. W.; BOTHA, F. C. Purification and characterisation of fructokinase from the culm of sugarcane. **Plant Science**, v. 167, n. 3, p. 645–654, 2004.

HU, D.G. et al. Glucose Sensor MdHXK1 Phosphorylates and Stabilizes MdbHLH3 to Promote Anthocyanin Biosynthesis in Apple. **PLoS genetics**, v. 12, n. 8, p. e1006273, ago. 2016.

HU, W. et al. New insights into the evolution and functional divergence of the SWEET family in *Saccharum* based on comparative genomics. **BMC Plant Biology**, v. 18, n. 1, 7 nov. 2018.

HU, X. et al. Genome-Wide Analysis of the Trehalose-6-Phosphate Synthase (TPS) Gene Family and Expression Profiling of ScTPS Genes in Sugarcane. **Agronomy**, v. 10, n. 7, p. 969, jul. 2020.

HUERTA-CEPAS, J. et al. EGGNOG 4.5: A hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. **Nucleic Acids Research**, v. 44, n. D1, p. D286—-D293, nov. 2016.

INGARGIOLA, C. et al. The Plant Target of Rapamycin: A Conduc TOR of Nutrition and Metabolism in Photosynthetic Organisms. **Genes**, v. 11, n. 11, p. E1285, 29 out. 2020.

JACINTO, E. et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. **Nature Cell Biology**, v. 6, n. 11, p. 1122–1128, nov. 2004.

JACKSON, P. A. Breeding for improved sugar content in sugarcane. **Field Crops Research**, Sugarcane physiology:Integrating from cell to crop to advance sugarcane production. v. 92, n. 2, p. 277–290, 14 jun. 2005.

JAISWAL, D. et al. Brazilian sugarcane ethanol as an expandable green alternative to crude oil use. **Nature Climate Change**, v. 7, n. 11, p. 788–792, 1 nov. 2017.

JAMSHEER K, M.; JINDAL, S.; LAXMI, A. Evolution of TOR–SnRK dynamics in green plants and its integration with phytohormone signaling networks. **Journal of Experimental Botany**, v. 70, n. 8, p. 2239–2259, 15 abr. 2019.

JOHNSON, L. N.; LEWIS, R. J. Structural basis for control by phosphorylation. **Chemical Reviews**, v. 101, n. 8, p. 2209–2242, ago. 2001.

JUNIOR, N. N. et al. Expression Analysis of Two Genes Coding for Trehalose-6-Phosphate Synthase (TPS), in Sugarcane (*Saccharum* spp.) under Water Stress. **American Journal of Plant Sciences**, v. 2013, 16 dez. 2013.

KANO, A. et al. The rare sugar D-allose acts as a triggering molecule of rice defence via ROS generation. **Journal of Experimental Botany**, v. 64, n. 16, p. 4939–4951, nov. 2013.

KARVE, A. et al. Expression and evolutionary features of the hexokinase gene family in Arabidopsis. **Planta**, v. 228, n. 3, p. 411–425, ago. 2008.

KARVE, A.; MOORE, B. D. Function of Arabidopsis hexokinase-like1 as a negative regulator of plant growth. **Journal of Experimental Botany**, v. 60, n. 14, p. 4137–4149, 2009.

KELLY, G. et al. Hexokinase mediates stomatal closure. **The Plant Journal: For Cell and Molecular Biology**, v. 75, n. 6, p. 977–988, set. 2013.

KELLY, G. et al. Guard-Cell Hexokinase Increases Water-Use Efficiency Under Normal and Drought Conditions. **Frontiers in Plant Science**, v. 10, p. 1499, 2019.

KHAN, Q. et al. Transcriptomic exploration of a high sucrose mutant in comparison with the low sucrose mother genotype in sugarcane during sugar accumulating stage. **GCB Bioenergy**, v. 13, n. 9, p. 1448–1465, 2021.

KOCH, K. E. Carbohydrate-modulated gene expression in plants. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 47, n. 1, p. 509–540, 1996.

KULIK, A. et al. SnRK2 Protein Kinases—Key Regulators of Plant Response to Abiotic Stresses. **OMICS : a Journal of Integrative Biology**, v. 15, n. 12, p. 859–872, dez. 2011.

LASTDRAGER, J.; HANSON, J.; SMEEKENS, S. Sugar signals and the control of plant growth and development. **Journal of Experimental Botany**, v. 65, n. 3, p. 799–807, 2014.

LI, C. et al. Molecular Characterization and Co-expression Analysis of the SnRK2 Gene Family in Sugarcane (*Saccharum officinarum* L.). **Scientific Reports**, v. 7, n. 1, p. 17659, 15 dez. 2017a.

LI, H. W. et al. Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. **Planta**, v. 234, n. 5, p. 1007–1018, nov. 2011.

LI, L.; SHEEN, J. **Dynamic and diverse sugar signaling Current Opinion in Plant Biology** Elsevier Ltd, 2016.

LI, X. et al. Differential TOR activation and cell proliferation in Arabidopsis root and shoot apexes. **Proceedings of the National Academy of Sciences of the United States of America**, v. 114, n. 10, p. 2765–2770, 7 mar. 2017b.

LIN, C.-R. et al. SnRK1A-Interacting Negative Regulators Modulate the Nutrient Starvation Signaling Sensor SnRK1 in Source-Sink Communication in Cereal Seedlings under Abiotic Stress. **The Plant Cell**, v. 26, n. 2, p. 808–827, 1 fev. 2014.

LU, C.-A. et al. The SnRK1A Protein Kinase Plays a Key Role in Sugar Signaling during Germination and Seedling Growth of Rice. **The Plant Cell**, v. 19, n. 8, p. 2484–2499, ago. 2007.

LUNN, J. E. Gene families and evolution of trehalose metabolism in plants. **Functional plant biology: FPB**, v. 34, n. 6, p. 550–563, jun. 2007.

LUNN, J. E. et al. Trehalose metabolism in plants. **The Plant Journal**, v. 79, n. 4, p. 544–567, ago. 2014.

LUNN, J. E.; MACRAE, E. New complexities in the synthesis of sucrose. **Current Opinion in Plant Biology**, v. 6, n. 3, p. 208–214, jun. 2003.

MA, P. et al. Comparative analysis of sucrose phosphate synthase (SPS) gene family between *Saccharum officinarum* and *Saccharum spontaneum*. **BMC Plant Biology**, v. 20, n. 1, p. 422, 14 set. 2020.

MAHFOUZ, M. M. et al. Arabidopsis TARGET OF RAPAMYCIN interacts with RAPTOR, which regulates the activity of S6 kinase in response to osmotic stress signals. **The Plant Cell**, v. 18, n. 2, p. 477–490, fev. 2006.

MARGALHA, L.; CONFRARIA, A.; BAENA-GONZÁLEZ, E. SnRK1 and TOR: modulating growth-defense trade-offs in plant stress responses. **Journal of Experimental Botany**, v. 70, n. 8, p. 2261–2274, 15 abr. 2019.

MARTÍNEZ-NOËL, G. M. A.; TOGNETTI, J. A. Chapter 22 - Sugar Signaling Under Abiotic Stress in Plants. In: AHMAD, P. et al. (Eds.). . **Plant Metabolites and Regulation Under Environmental Stress**. [s.l.] Academic Press, 2018. p. 397–406.

MARTINS, M. C. M. et al. Feedback inhibition of starch degradation in Arabidopsis leaves mediated by trehalose 6-phosphate. **Plant Physiology**, v. 163, n. 3, p. 1142–1163, nov. 2013.

MCCORMICK, A. J.; CRAMER, M. D.; WATT, D. A. Sink strength regulates photosynthesis in sugarcane. **The New Phytologist**, v. 171, n. 4, p. 759–770, 2006.

MCCORMICK, A. J.; CRAMER, M. D.; WATT, D. A. Differential Expression of Genes in the Leaves of Sugarcane in Response to Sugar Accumulation. **Tropical Plant Biology**, v. 1, n. 2, p. 142–158, 1 jun. 2008.

MCKIBBIN, R. S. et al. Production of high-starch, low-glucose potatoes through over-expression of the metabolic regulator SnRK1. **Plant Biotechnology Journal**, v. 4, n. 4, p. 409–418, 2006.

MENAND, B. et al. Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. **Proceedings of the National Academy of Sciences**, v. 99, n. 9, p. 6422–6427, 30 abr. 2002.

MERRITT, H.; BARRAGÁN-OCAÑA, A. The impact of market factors on the development of eco-friendly energy technologies: the case of bioethanol. **Clean Technologies and Environmental Policy**, 30 out. 2021.

MING, R. et al. Sugarcane Improvement through Breeding and Biotechnology. In: **Plant Breeding Reviews**. [s.l.] John Wiley & Sons, Ltd, 2005. p. 15–118.

MOHAN, C.; EASTERLING, M.; YAU, Y.Y. Gene Editing Technologies for Sugarcane Improvement: Opportunities and Limitations. **Sugar Tech: An**

International Journal of Sugar Crops & Related Industries, p. 1–17, 15 out. 2021.

MOORE, B. et al. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. **Science (New York, N.Y.)**, v. 300, n. 5617, p. 332–336, 11 abr. 2003.

MOREAU, M. et al. Mutations in the Arabidopsis Homolog of LST8/GβL, a Partner of the Target of Rapamycin Kinase, Impair Plant Growth, Flowering, and Metabolic Adaptation to Long Days. **The Plant Cell**, v. 24, n. 2, p. 463–481, fev. 2012.

MUBEEN, U. et al. Target of Rapamycin Inhibition in *Chlamydomonas reinhardtii* Triggers de Novo Amino Acid Synthesis by Enhancing Nitrogen Assimilation. **The Plant Cell**, v. 30, n. 10, p. 2240–2254, 1 out. 2018.

NOËL, G. M.; TOGNETTI, J. A.; PONTIS, H. G. Protein kinase and phosphatase activities are involved in fructan synthesis initiation mediated by sugars. **Planta**, v. 213, n. 4, p. 640–646, ago. 2001.

NUKARINEN, E. et al. Quantitative phosphoproteomics reveals the role of the AMPK plant ortholog SnRK1 as a metabolic master regulator under energy deprivation. **Scientific Reports**, v. 6, n. 1, p. 31697, 22 ago. 2016.

O'LEARY, B. M. et al. Metabolite Regulatory Interactions Control Plant Respiratory Metabolism via Target of Rapamycin (TOR) Kinase Activation. **The Plant Cell**, v. 32, n. 3, p. 666–682, 1 mar. 2020.

O'NEILL, B. P. et al. RNAi-mediated abrogation of trehalase expression does not affect trehalase activity in sugarcane. **SpringerPlus**, v. 1, n. 1, p. 1–6, 2012.

PAPINI-TERZI, F. S. et al. Sugarcane genes associated with sucrose content. **BMC Genomics**, v. 10, n. 1, p. 120, 21 mar. 2009.

PAUL, M. J.; FOYER, C. H. Sink regulation of photosynthesis. **Journal of Experimental Botany**, v. 52, n. 360, p. 1383–1400, 1 jul. 2001.

PEIXOTO, B. et al. Impact of the SnRK1 protein kinase on sucrose homeostasis and the transcriptome during the diel cycle. **Plant Physiology**, v. 187, n. 3, p. 1357–1373, 3 nov. 2021.

POLGE, C. et al. β-subunits of the SnRK1 complexes share a common ancestral function together with expression and function specificities; physical interaction with nitrate reductase specifically occurs via AKINβ1-subunit. **Plant Physiology**, v. 148, n. 3, p. 1570–1582, 2008.

POLGE, C.; THOMAS, M. SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control? **Trends in Plant Science**, v. 12, n. 1, p. 20–28, jan. 2007.

PRIJI, P. J.; HEMAPRABHA, G. Sugarcane Specific Drought Responsive Candidate Genes Belonging to ABA Dependent Pathway Identified from Basic Species Clones of *Saccharum* sp. and *Erianthus* sp. **Sugar Tech**, v. 17, n. 2, p. 130–137, 1 jun. 2015.

QIN, C.-X. et al. Identification of proteins and metabolic networks associated with sucrose accumulation in sugarcane (*Saccharum* spp. interspecific hybrids). **Journal of Plant Interactions**, v. 16, n. 1, p. 166–178, 1 jan. 2021.

RADCHUK, R. et al. Sucrose non-fermenting kinase 1 (SnRK1) coordinates metabolic and hormonal signals during pea cotyledon growth and differentiation. **The Plant Journal: For Cell and Molecular Biology**, v. 61, n. 2, p. 324–338, jan. 2010.

RIGGI, M. et al. TORC2 controls endocytosis through plasma membrane tension. **The Journal of Cell Biology**, v. 218, n. 7, p. 2265–2276, 1 jul. 2019.

ROBERTS, D. J. et al. Hexokinase-II positively regulates glucose starvation-induced autophagy through TORC1 inhibition. **Molecular Cell**, v. 53, n. 4, p. 521–533, 20 fev. 2014.

RODRÍGUEZ-SAAVEDRA, C. et al. Moonlighting Proteins: The Case of the Hexokinases. **Frontiers in Molecular Biosciences**, v. 8, p. 701975, 2021.

ROLLAND, F.; BAENA-GONZALEZ, E.; SHEEN, J. Sugar sensing and signaling in plants: Conserved and Novel Mechanisms. **Annual Review of Plant Biology**, v. 57, n. 1, p. 675–709, 1 jun. 2006.

SALAZAR-DÍAZ, K. et al. TOR senses and regulates spermidine metabolism during seedling establishment and growth in maize and Arabidopsis. **iScience**, v. 24, n. 11, p. 103260, 19 nov. 2021.

SALEM, M. A. et al. RAPTOR Controls Developmental Growth Transitions by Altering the Hormonal and Metabolic Balance. **Plant Physiology**, v. 177, n. 2, p. 565–593, 1 jun. 2018.

SAROWAR, S. et al. A role of hexokinases in plant resistance to oxidative stress and pathogen infection. **Singmul Hakhoe chi. The Korean journal of botany**, v. 51, n. 5, p. 341–346, 30 ago. 2008.

SCHEPETILNIKOV, M. et al. TOR and S6K1 promote translation reinitiation of uORF-containing mRNAs via phosphorylation of eIF3h. **The EMBO journal**, v. 32, n. 8, p. 1087–1102, 17 abr. 2013.

SCHLUEPMANN, H. et al. Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in *Arabidopsis thaliana*. **Proceedings of the National Academy of Sciences of the United States of America**, v. 100, n. 11, p. 6849–6854, maio 2003.

SCORTECCI, K. C. et al. **Challenges, Opportunities and Recent Advances in Sugarcane Breeding**. [s.l.] IntechOpen, 2012.

SERFONTEIN, J. et al. Evolution of the TSC1/TSC2-TOR Signaling Pathway. **Science Signaling**, 29 jun. 2010.

SHI, Y. et al. Comparative Analysis of SUS Gene Family between *Saccharum officinarum* and *Saccharum spontaneum*. **Tropical Plant Biology**, v. 12, n. 3, p. 174–185, 1 set. 2019.

SINGH, R. et al. Transcriptomic analysis of transgressive segregants revealed the central role of photosynthetic capacity and efficiency in biomass accumulation in sugarcane. **Scientific Reports**, v. 8, n. 1, p. 4415, 13 mar. 2018.

SMITH, A. M.; STITT, M. Coordination of carbon supply and plant growth. **Plant, Cell & Environment**, v. 30, n. 9, p. 1126–1149, set. 2007.

SONG, Y. et al. The crosstalk between Target of Rapamycin (TOR) and Jasmonic Acid (JA) signaling existing in Arabidopsis and cotton. **Scientific Reports**, v. 7, p. 45830, 4 abr. 2017.

SONG, Y. et al. Contributions of TOR Signaling on Photosynthesis. **International Journal of Molecular Sciences**, v. 22, n. 16, p. 8959, 20 ago. 2021.

SORMANI, R. et al. *Saccharomyces cerevisiae* FKBP12 binds *Arabidopsis thaliana* TOR and its expression in plants leads to rapamycin susceptibility. **BMC Plant Biology**, v. 7, p. 26, 1 jun. 2007.

SOTELO, R. et al. Coordination of cell growth and cell division in maize (*Zea mays* L.) relevance of the conserved TOR signal transduction pathway. **In**

Vitro Cellular & Developmental Biology - Plant, v. 46, n. 6, p. 578–586, 1 dez. 2010.

SOTO-BURGOS, J.; BASSHAM, D. C. SnRK1 activates autophagy via the TOR signaling pathway in *Arabidopsis thaliana*. **PloS One**, v. 12, n. 8, p. e0182591, 2017.

STITT, M.; LUNN, J.; USADEL, B. Arabidopsis and primary photosynthetic metabolism - more than the icing on the cake. **The Plant Journal: For Cell and Molecular Biology**, v. 61, n. 6, p. 1067–1091, mar. 2010.

SUN, L. et al. Ribosomal protein S6 kinase1 coordinates with TOR-Raptor2 to regulate thylakoid membrane biosynthesis in rice. **Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids**, v. 1861, n. 7, p. 639–649, 1 jul. 2016.

SUN, M.H. et al. The Glucose Sensor MdHXK1 Phosphorylates a Tonoplast Na+/H+ Exchanger to Improve Salt Tolerance. **Plant Physiology**, v. 176, n. 4, p. 2977–2990, abr. 2018.

The impact of disasters and crises on agriculture and food security: 2021. [s.l.] FAO, 2021.

THIRUGNANASAMBANDAM, P. P.; HOANG, N. V.; HENRY, R. J. The Challenge of Analyzing the Sugarcane Genome. **Frontiers in Plant Science**, v. 9, 2018.

VAN DAM, T. J. P. et al. Evolution of the TOR Pathway. **Journal of Molecular Evolution**, v. 73, n. 3, p. 209–220, 2011.

VILELA, M. DE M. et al. Analysis of Three Sugarcane Homo/Homeologous Regions Suggests Independent Polyploidization Events of *Saccharum officinarum* and *Saccharum spontaneum*. **Genome Biology and Evolution**, v. 9, n. 2, p. 266–278, 1 fev. 2017.

WACLAWOVSKY, A. J. et al. Sugarcane for bioenergy production: an assessment of yield and regulation of sucrose content. **Plant Biotechnology Journal**, v. 8, n. 3, p. 263–276, abr. 2010.

WAN, H. et al. Evolution of Sucrose Metabolism: The Dichotomy of Invertases and Beyond. **Trends in Plant Science**, v. 23, n. 2, p. 163–177, fev. 2018.

WANG, J. et al. Carbon partitioning in sugarcane (*Saccharum* species). **Frontiers in Plant Science**, v. 4, p. 201, 2013.

WANG, J. et al. Characteristics, Expression Pattern and Intracellular Localisation of Sugarcane Cytoplasmic Hexokinase Gene ShHXK8. **Sugar Tech**, v. 21, n. 6, p. 909–916, 1 dez. 2019.

WEISTE, C. et al. The Arabidopsis bZIP11 transcription factor links low-energy signaling to auxin-mediated control of primary root growth. **PLOS Genetics**, v. 13, n. 2, p. e1006607, 3 fev. 2017.

WILLIAMS, S. P. et al. Regulation of Sucrose non-Fermenting Related Kinase 1 genes in Arabidopsis thaliana. **Frontiers in Plant Science**, v. 5, 2014.

WU, Y. et al. Integration of nutrient, energy, light, and hormone signaling via TOR in plants. **Journal of Experimental Botany**, v. 70, n. 8, p. 2227–2238, 15 abr. 2019.

WULLSCHLEGER, S.; LOEWITH, R.; HALL, M. N. TOR signaling in growth and metabolism. **Cell**, v. 124, n. 3, p. 471–484, 10 fev. 2006.

WURZINGER, B. et al. The SnRK1 Kinase as Central Mediator of Energy Signaling between Different Organelles. **Plant Physiology**, v. 176, n. 2, p. 1085–1094, fev. 2018.

XIONG, Y. et al. Glucose–TOR signaling reprograms the transcriptome and activates meristems. **Nature**, v. 496, n. 7444, p. 181–186, abr. 2013.

XIONG, Y.; SHEEN, J. Rapamycin and glucose-target of rapamycin (TOR) protein signaling in plants. **The Journal of Biological Chemistry**, v. 287, n. 4, p. 2836–2842, 20 jan. 2012.

YADAV, U. P. et al. The sucrose-trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signaling by Tre6P. **Journal of experimental botany**, v. 65, n. 4, p. 1051–1068, mar. 2014.

YANG, H. L. et al. Molecular Evolution of Trehalose-6-Phosphate Synthase (TPS) Gene Family in Populus, Arabidopsis and Rice. **PLOS ONE**, v. 7, n. 8, p. e42438, ago. 2012.

ZHANG, Z. et al. Isolation, structural analysis, and expression characteristics of the maize (*Zea mays* L.) hexokinase gene family. **Molecular Biology Reports**, v. 41, n. 9, p. 6157–6166, 2014.

ZHOU, M.-L. et al. Trehalose Metabolism-Related Genes in Maize. **Journal of Plant Growth Regulation**, v. 33, n. 2, p. 256–271, 1 jun. 2014.

ZHU, Y. J.; KOMOR, E.; MOORE, P. H. Sucrose Accumulation in the Sugarcane Stem Is Regulated by the Difference between the Activities of Soluble Acid Invertase and Sucrose Phosphate Synthase. **Plant Physiology**, v. 115, n. 2, p. 609–616, 1 out. 1997.

FINAL CONSIDERATIONS AND PERSPECTIVES

Final considerations and perspectives

Sugarcane is an important crop in the Brazilian agriculture scenario, and over the years, it has played a new role in bioenergy strategies. One of the alternatives to boost the use of bioethanol is to increase productivity without expanding the planted area. To reach this goal, it is essential to have a holistic understanding of the internal machinery, which synergistically depends on metabolic reaction rates, water/nutrient availability, and cellular and developmental programs that connect sugar sensing and signaling-related genes. However, the complexity of sugar perception and signaling pathways have not been explored in this species, which accumulates impressive amounts of sucrose. One of the significant challenges for elucidating most of the biological mechanisms in sugarcane is the identification of target genes related to the sugar-sensing mechanism. However, despite the scientific community's efforts, the coverage of the sugarcane genome remains incomplete, functioning as a bottleneck to understanding how sugarcane can accumulate such a high amount of sucrose in culm cells. Furthermore, this makes it difficult to take the first steps towards the biotechnological improvement of sugarcane.

Nevertheless, research in sugarcane has advanced in the later years due to the use of high-throughput techniques such as RNAseq, which allowed the refinement of analyzes, making it more tangible to access more precise data from the different physiological, biochemical, and molecular processes such as partition and allocation of C in this culture. As explored in the first and second chapters of this thesis, we used seven genome and transcriptome sugarcane databases to identify the sequences and evolutionary relationship of sugar signaling-related targets (HXK, TORC1, SnRK1α, and enzymes from trehalose metabolism) through the integration of distinct analyses such as protein structure, phylogeny, and functional protein domains characterization.

The results described in chapter 1, dedicated explicitly to the T6P pathway, revealed 71 full-length putative TPS, 93 TPP, and 3 TRE that contained all conservative domains related to their respective protein families. These sequences were categorized into different phylogenetic groups according to each family. Most of the sequences from one sugarcane transcriptomic dataset showed variable expression levels in leaves. Furthermore, TPS Class I has specific *N*-glycosylation sites inserted in conserved motifs and contains catalytic

and binding residues in its TPS domain. Some of these residues are mutated in TPS Class II members, implicating in loss of enzyme activity.

In chapter 2, we found 11 sequences of TOR, 23 of RAPTOR, 25 of LST8, 50 of SnRK1α, 69 of HXK, and 9 of HXK-Like that also possess all their conservative domains. Specifically for TORC1, our work is the pioneer in recovering sequences from all the members of the complex (TOR, RAPTOR, and LST8). Most of the sequences from one sugarcane transcriptomic dataset presented variable expression levels in leaves. Sequences of HXK were grouped in the different clades of phylogenetic trees. Besides that, the highest catalytic activity of the HXK enzyme was detected in culm in month 01, which may indicate an upregulation for plant growth during bud resprouting.

In summary, with the results of the first two chapters, it was possible to obtain baseline information to elucidate the mechanism of action of the targets and for biotechnological applications. Our data:

1. Helped to elucidate crucial differences between catalytic and regulatory TPS (catalytic TPS forms T6P, a homeostatic regulator of sucrose levels);
2. Detected important regulatory points of the HXK enzyme (month 01 in culm), in which it can integrate signaling networks to control growth and development in response to environmental inputs; and
3. Recovered high-quality sequences that can be used for obtaining more accurate expression values for each enzyme/isoform and future genetic transformations in sugarcane.

A comparison of the total number of identified sequences of sugar sensing pathways in sugarcane (our results) with other organisms is shown in Fig. 1.

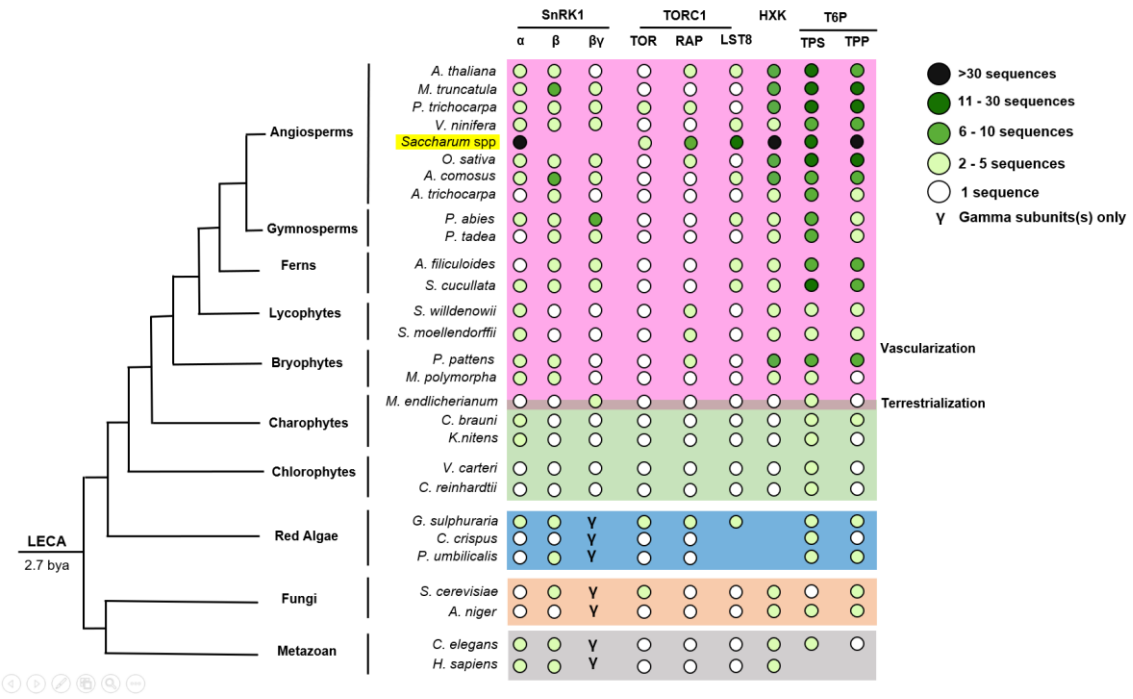

Figure 1. Overview of the sugar sensing genes in sugarcane and other organisms. The occurrence and number of homolog(s) in each species are shown by a circle with different colors that are described in the figure legend. The numbers of sequences in sugarcane were identified by EggNOG as described in chapters one e two. For the other organisms, the number of sequences was obtained from the literature (Fichtner et al., 2021). The tree shows the most commonly accepted phylogenetic relationships among the groups represented and LECA stands for the last eukaryotic common ancestor. Figure adapted from (Fichtner et al., 2021).

In chapter 3, the experiment published by de Souza et al. (2018) was expanded to include amino acids, polyamines, and the HXK enzymatic activity. The results indicate that sugarcane metabolism systematically changes sharply during the first three months of development. When analyzing the metabolism of amino acids and polyamines under field conditions in developing sugarcane (01, 03, 06, and 12 months), there is more significant variation in the total amino acid content in the leaf over the months, while in the stem the first month has the highest contents, acting as a potent sink of these metabolites. Polyamines are more abundant in leaves in all months, with a decrease in month 12, while in the culm, the lowest amounts were found in month 6. Putrescine was the most abundant polyamine in the leaf in month 1, possibly acting as a growth promoter. The polyamines and amino acids concentrations are organ-specific, and the stem showed a more coordinated metabolism than the leaf.

The data generated in this thesis allows a systemic analysis that includes different organization levels: physiological, biochemical, metabolic, and molecular.

The first two chapters, in which one of the objectives was to identify sequences and characterize protein domains, served as the basis for constructing a Targeted Seq (TAS). Unlike other more conventional techniques, such as RNA-seq, to build the TAS, it is necessary to select the targets in advance to construct pairs of targeted primers that will be added before the amplification to generate a unique signature for each sample. Thus, the identified sugarcane sequences were used to build a panel of selected genes that will be amplified before sequencing, allowing the identification of rare isoforms presented in a given exon.

All pathways/sequences mentioned in this thesis were selected, plus the downstream targets of some sensors, such as bZIP11 (a target of SnRK1) and specific targets of TORC1 as ATG1a, which is autophagy-related and protein ribosomal protein as RPS5. Because sugarcane does not have a full-sequenced genome, additional steps were necessary to allow the amplification of genes. These included identifying UTR regions (necessary for constructing primers) and analyzing whether the sequences had expression values in sugarcane (in addition to what was used in chapters 1 and 2 for SP80-3280). Thus, all the sequences used in these analyses were sent to the panel's construction (TSA) and will be sequenced using RNA templates from leaves and culms of sugarcane grown in the field. This thesis provides access to all these sequences for the sugarcane cultivar SP80-3280.

With this in hand, it will be possible to carry out a deeper integrative analysis considering the physiology, biochemistry, and transcripts, which constitute a regulation at different levels among several steps of sugarcane development. Because we have biological materials from the field experiment (De Souza et al. 2018), the TSA analysis will help to evaluate the dynamic of gene expression during the entire cycle of sugarcane growth and development. It is expected that such integration will enable to exploration sugar-mediated responses through sugar sensing and signaling during sugarcane development. This is crucial to provide new insights into manipulating metabolism to improve sucrose accumulation and plant growth performance in the context of environmental stresses that are expected to rise with climate change.