Unravelling the Potential Long-Distance Sugar Signalling in Plant Growth and Development.

A literature review by: Konstantina Pantazi

Student Number: 7370490

Examiner: Dr. Kaisa Kajala

Supervisor: Dr. Kaisa Kajala

Utrecht University   UU Plants

Acknowledgements

Abstract

The orchestration of a plant's growth and development is dependent on an complex web of signalling pathways, with sugars emerging as a central player. Plants are autotrophic organisms which means that they rely on sugars that are derived from photosynthesis, which are crucial for the plant's growth. Recent studies have emphasised the evolution of mechanisms that allow the plant to perceive sugars, revealing their dual role in metabolism and signalling. Sugar signalling allows the plants to integrate external as well as internal cues, maintaining nutrient homeostasis and respond to stresses. There is evidence of many environmental stresses that utilise sugar signals. Some examples are drought, salt and cold stress. Short-distance sugar signalling mechanisms have been well documented; however, long-distance sugar signalling remains largely unexplored. The lack of studies exploring long-distance sugar signals could be attributed to the fact that signalling networks are very complex and are often interconnected with different signalling networks making it difficult to study especially when talking about long-distance signalling. Sugars are dynamic molecules which means that changes in their levels is rapid when responding to changes in environmental stimuli. A lot of these signalling sugar pathways work intracellularly so putting them in the context of long-distance signalling makes it a lot more complicated. In this literature review I researched the established short-distance sugar signalling pathways, shed light on the mechanisms that orchestrate physiological responses in plants and investigated if there is any evidence of these signalling pathways working in the long-distance context. Also, in this review I suggest that the crosstalk between sugar signalling pathways and other signalling pathways and molecules could contribute to long-distance sugar signalling.

Layman's Summary

Plant have developed many complex ways to help them communicate information. In order to survive, plant have to photosynthesise, which is the process in which plant make food using light from the sun. Plants capture the sunlight, absorb water from their roots and take carbon dioxide form the air, resulting in the production of glucose and the release of oxygen back into the atmosphere. Glucose is a sugar and the main energy source for plants. Just like how our bodies produce signals to be able to response to different changes, plants do the same. Plants have developed these signalling networks to communicate and respond to their environment in an effective way. Sugar signalling refers to the communication system where sugars have a role as a signalling molecule beyond just being mere sources of energy. The networks allow plants to adapt to different changes like light, temperature and nutrient levels. Sugars have been found to act as messengers, relaying information about these changes in the plant's environment. These signalling networks allow the plant to coordinate responses in order to help the plant adapt to changing circumstances and potentially enhancing the plant's resilience. These networks allow them to adapt to changes, such as light, temperature, and nutrient levels. Signalling helps plants coordinate various processes like growth, defense against pests, and the allocation of resources. Essentially, these networks enable plants to "sense" their surroundings and make adjustments to survive and thrive in different conditions. A lot of research can be found about how these networks work in short distance, which means within the plant's cells or over small distances but little is known about sugar signalling when it comes to relaying information from the one end of the plant to the other.

Introduction

Sugars are fundamental molecules for supporting life on Earth. Plants are autotrophic organisms as they photosynthesise in order to generate carbon skeletons, producing sugars which are crucial for structural components as well as providing energy for the plant (Wang et al., 2020). Plants have evolved complex mechanisms to sense different sugars like sucrose, trehalose and hexoses (Sakr et al. 2018). Traditionally, sugars are known to be components of metabolic pathways, however, it is now being established that sugars can act as signalling molecules. Sugar signalling is important for plants as they have to integrate internal as well as external cues, maintain nutrient homeostasis, mediate developmental programs and orchestrate responses to biotic and abiotic stresses (Roland et al., 2002). Some of these signalling pathways are ancient and have originated in primitive eukaryotes even before plants, animals and fungi diverged. Recent studies have revealed a correlation between growth rate and overall sugar levels, and it has been suggested that growth rate is controlled by metabolic signals rather than the availability of these metabolic substrates (Sakr et al. 2018). By this I mean that while the availability of key nutrients and fixed carbon is undoubtedly crucial, the control mechanism for growth lies more in the signals that are produced by these metabolic processes rather than just the sheer abundance of the metabolic substrates. Therefore, sugars are not only an energy source that have a role in metabolism but also act as important signals for coordinating the plant's growth and development with the everchanging environmental conditions.

Before setting off to explore all the different sugar signalling pathways it's important to establish what signalling is, what makes a signal, and what comprises a signalling pathway. In order for plants to be able to respond to environmental stimuli, plants employ various signal transduction pathways, series of coordinated events that occur in responses to a specific signal. These pathways allow the transmission of information from the site of signal perception to the target cellular components where a response is triggered (Mulligan et al., 1997). They often involve a cascade of molecular interactions and changes in gene expression that ultimately lead to specific biochemical and physiological responses in the plant. In the context of signalling, the signal is a molecular cue or stimulus that initiates a response in the plant. Signals can be physical stimuli, hormones or secondary messengers. To meet the definition of a signal, it needs to be recognised by specific receptors in the plant cells, which then transmit the signal though a series of molecular events in the signalling pathway. In other words, for a molecule or a stimulus to be able to act as a signal depends on its capacity to convey information and elicit a response within the plant's molecular and cellular processes (Alberts et al.,2002).

Carbohydrates that are derived from the shoot are transported to the sink tissues (roots, tubers, fruit, seeds etc.) through the phloem, together with other metabolites and signalling molecules (Turgeon and Wolf, 2009). As mentioned before, carbohydrates have a role as intermediate substrates for metabolism, providing carbon backbones and energy necessary for the growth and development of sink tissues. In their role as signalling molecules, carbohydrates can either promote or inhibit growth. Some of the signalling cascades that promote growth involve trehalose 6-phosphate (T6P) signal and hexokinase Glc sensor. Examples of growth inhibiting signals involve SNF1-Related Protein Kinase 1(SnRK1) and bZIP transcription factors (Smeekens et al., 2010). I will expand on each example later in this literature review. The most common carbohydrate found in the phloem is sucrose, however, a sucrose-specific sensor has not yet been found although it has been shown that there are sucrose dependent signalling pathways (Wind et al., 2010). Short-distance signalling has been described for several sugars; however long-distance signalling remains an unexplored area.

Photoautrophy or the production of food through light presents significant regulatory challenges, especially in multicellular plants. Carbon fixation is confined in specific plant cells that change in number and location during the plant's development. The cells have to share their metabolic products with the distant parts of the plant, necessitating a functional equilibrium in order to maintain a metabolic balance. Throughout the

day plants have to balance metabolic demands and cope with the daily fluctuations of carbon fixation rates in the whole plant. During the daytime, growth is dependent on the sugars produced through photosynthesis while during the night, growth depends on the starch stored during the day (Wiese et al., 2007). Understanding how plants communicate carbon status over long distance is a missing piece in comprehending the plants' integrate systems.

My hypothesis is that while there may not be direct evidence confirming the existence of long-distance sugar signals, the interplay of already established sugar signalling pathways, coupled with their interactions with other signalling pathways, contributes significantly to the potential phenomenon of long-distance sugar signalling in plants. In this review I wanted to explore the established sugar signalling pathways that have been well-characterised as well as crosstalk of these pathways with other signalling pathways and if there could be evidence that these could functionally provide long-distance sugar signalling. I especially set out to address the following questions:

- **Question 1:** Do the well-established sugar signalling pathways have a role in long distance sugar signalling as well?
- **Question 2:** Does the crosstalk of sugar signalling pathways and other signalling pathways attribute to long-distance sugar signalling?

Disaccharide Sugar Signalling

Sucrose

Motivated by the essential role of sucrose in plant physiology and its known signalling functions, I first explored the possibility of sucrose's involvement in long-distance sugar signalling. Plants acquire carbon from atmospheric carbon dioxide producing sugar phosphates during photosynthetic assimilation for plant growth and development (Chen et al., 2012; Stadler et al., 2015; Gottwald et al., 2000). Excess photo assimilates are stored in the form of starch and low levels of sucrose undergo turnover during the day and storage at night (Chen et al., 2012; Stadler et al., 2015; Gottwald et al., 2000). Enhanced photosynthesis leads to an increase in sucrose phloem loading, maintaining balance between the source and sink tissues under favourable conditions (Chen et al., 2012).

Sucrose is one of the main transported sugars but also has a dual role as it also acts as a signalling entity. This was proven by many experiments that revealed that non-metabolised sucrose analogs can mimic the effects of sucrose while hexose derivative like fructose and glucose had very low efficiency (Chiou et al., 1998; Loreti et al., 2000 et al.; Fernie et al., 2001; Teng et al., 2005). The proton-coupled sucrose symporter called Sucrose Transporter (SUT) mediates phloem loading which is a crucial part of assimilate partitioning (Giaquinta et al., 1983; Bush et al., 1992; Riessmeier et al. 1994). The activity of symporters is regulated by the sucrose-dependent signalling pathway in response to the sucrose levels in the leaves. This regulation is specific to sucrose and is dependent on its concentration which is linked to change in the transcript abundance and vice versa. The alanine symporter activity and facilitated glucose transport remains unaffected by sucrose, which points to the specificity to the sucrose symporter (Chiou et al., 1998). Changes in gene expression are commonly sugar dependent for this phenomenon, integrating cellular responses to changing resource allocation and utilisation. The messenger RNA levels of the symporter decrease with sucrose transport activity which could suggest regulation at the transcriptional level. Sucrose-dependent signalling pathway could involve the transcriptional regulation and protein turnover, impacting symporter gene expression and protein synthesis (Chiou et al., 1998). A well-known example is the down regulation of BvSUT1 (Beta vulgaris Sucrose Transporter 1) which is sucrose specific. Beta vulgaris Sucrose Transporter (BvSUT1), a gene encoding a sucrose transporter, specifically in response to sucrose, not hexoses, highlights the control mechanisms in

plants. This regulation occurs at the phloem loading site, influencing the distribution of photo-assimilates between source and sink tissues. While the exact mechanism of sucrose perception remains elusive, studies suggest the involvement of proteins like AtSUT2/SUC3 and SUT4 in sugar signaling pathways. Downstream of sucrose sensing, calcium-related proteins and protein kinases are implicated in transmitting sucrose signals (Chiou et al., 1998).

Sucrose has emerged as a signalling entity as well as being an important metabolite in plants. This disaccharide, has great potential to also act as a sugar signal in long-distances as it is mobile and is able to travel easily through the phloem from its production site to wherever it is needed, even all the way down to the root system. It is also relatively stable, meaning that it doesn't break down so easily when it gets transported which makes me think that its signalling function can be maintained and extended over long-distances. It seems that sucrose doesn't sense changes in sugar levels itself, however, there is emerging evidence suggesting that it is involved in transcriptional regulation and protein turnover which affects protein synthesis and gene expression of symporters, therefore, further research could reveal the effect of sucrose in regulating the transcription and expression of other genes that are potentially involved in long-distance sugar signalling.

Trehalose-6-phosphate (T6P)

Intrigued by the central role of Trehalose-6-phosphate (T6P) in sugar signalling and its regulatory functions, I wanted to next explore its potential involvement in long-distance sugar signalling. Trehalose 6-phosphate (Tre6P) is a signal metabolite that regulates sucrose metabolism in plants which connects growth and development to metabolic status (Leyman et al., 2001). The interplay between sucrose and trehalose 6P (T6P) emerges as a critical regulatory mechanism in plants, impacting cell metabolism, growth, and responses to stress (Paul et al., 2008; Yadav et al., 2014; Figueroa et al., 2018; Fichtner et al., 2007). T6P, synthesized from UDP-glucose and glucose 6-phosphate, closely linked to sucrose levels, forming the basis of the Suc-T6P nexus model (Cabib et al., 1958). This model proposes T6P as both a signal and a negative feedback regulator of sucrose levels, ensuring optimal sucrose concentrations based on developmental stages and environmental cues (Figueroa et al., 2016). The intricate balance between sucrose and T6P is further supported by the observation that T6P levels mirror sucrose levels, underscoring the significance of these molecules in coordinating plant growth and development with carbon availability. The regulatory roles of T6P and sucrose are intertwined, suggesting a sophisticated network of sugar signaling pathways that orchestrate plant responses to varying physiological and environmental conditions. The complexity of these interactions underscores the need for further research to elucidate the complete sucrose signaling pathway and its impact on plant physiology. T6P also has been found to have a role in influencing the strength of the sink by regulating the expression of genes that have a role in sink growth and carbon metabolism. Sink tissues like roots and fruits have a high demand for assimilates. High T6P levels correlate with active growth and development while in low levels indicate a stunt in growth and a transition into reproductive stages (Figueroa et al., 2016).

T6P is a phosphorylated disaccharide precursor, involved in the synthesis of trehalose. The synthesis of trehalose in eukaryotes happens via T6P, the phosphorylated intermediate which is produced by Tre6P synthases (TPS). Trehalose-6-Phosphate Synthase 1 (TPS1) has an extra N-terminal domain which has an inhibitory function and its mainly localised in the vasculature, which positions T6P synthesis in a location where it is poised for signalling long-distance between source and sink tissues (Fichtner et al., 2020). There is a correlation between T6P levels and sucrose levels in source and sink (Zhang et al., 2009; O'Hara et al., 2012; Schluepmann et al., 2012), confirmed from sugar feeding experiments in *Arabidopsis*, and T6P is considered a signal reflecting sucrose status (Yadav et al.,2014). When the plant is experiencing nitrogen starvation, T6P is affected (Yadav et al.,2014) but the correlation of T6P with sucrose suggests that these changes are explained by altered sucrose levels. TPS1 enzymatic activity is negatively regulated by sucrose

and T6P is a negative feedback regulator, redirecting the carbon flux towards organic and amino acid synthesis (Yadav et al.,2014; Figueroa et al., 2016; Carillo et al., 2013). From the studies discussed above, T6P has many regulatory functions making T6P a potential key player in long-distance sugar signalling. The utilization of sucrose for the growth of developing tissues is significantly shaped by the opposing actions of two protein kinases: SUC-NON-FERMENTING-1-RELATED KINASE1 (SnRK1) and TARGET OF RAPAMYCIN (TOR) (more on these protein kinases will be discussed later in this review.). The interplay between T6P and SnRK1 in developing tissues has yet to be elucidated, encompassing both direct and indirect mechanisms, as well as displaying both positive and negative effects (Figueroa et al., 2016). A direct association between T6P and TOR has not been established to date, but more studies will help characterise and understand these relationship, possibly attributing evidence of long-distance sugar signalling (Figueroa et al., 2016).

While sucrose remains the primary long-distance sugar that is transported in the phloem, T6P is also mobile and is able to travel down the phloem which makes it a good candidate as long-distance sugar signal. T6P is also sensitive to changes in sugar levels, especially sucrose. This sensitivity could reflect its involvement in the overall sugar status of the plant. One of its regulatory roles is its influence on sink strength, therefore, his strong involvement in the below part of the plants gives promising evidence a long-distance sugar signal. More research should be done to elucidate the exact mechanisms in which T6P acts over long-distances.

Hexokinase Dependent Sugar Signalling

Next, I explored the role of hexokinase in sugar metabolism and its suggested involvement in sugar sensing as well as its potential contribution to long-distance sugar signalling. Hexokinase (HXK) is an enzyme that is involved in the first step of glycolysis, which is a central metabolic pathway in cells. HXK catalyses the phosphorylation of hexoses, like glucose, by using ATP to produce hexose-6-phosphate and ADP. This phosphorylation is a crucial step in trapping glucose within the cell, hence initiating metabolism (Barbier et al., 2021). In addition to its role in metabolism, it has been suggested that HXK has a role is sugar sensing and signalling, possessing both regulatory and catalytic functions.

Several studies have been conducted, revealing the physiological roles of HXK genes. This has been done by exposing the enzyme to exogenous sugars, modifying HXK expression in transgenic plants and analysing these mutants (Xiao et al., 2000). A study showed that maize protoplasts that were exposed to sugars, repressed photosynthetic gene expression, indicating sugar sensing (Jang and Sheen, 1994). The repression of photosynthetic genes by HXK is independent of downstream metabolism of glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P) (Jang and Sheen,1997; Jang et al., 1997), which supports a signalling role for HXK. Sugar sensing mediated by AtHXK1 involves a complex with VHA-B1 and RPT5B, possibly altering gene expression (Moore et al., 2003). As mentioned above HXK1 is a glucose sensor integrating nutrient and hormonal signal in order to control gene expression and plant growth in response to different environmental cues. A study by Cho et al., explored the mechanism in which HXK1 mediates glucose signalling. They revealed that two nuclear-specific HXK1 unconventional partners using proteomic and binary interactions screens. The two nuclear-specific partners were identified as vacuolar H+-ATPase (VHA-B1) and 19S regulatory particle of proteasome subunit (RPT5B). Chromatin immunoprecipitation and genetic analysis has suggested that nuclear HXK1 forms a glucose signalling complex core with VHA-B1 and RPT5B. This complex directly modulates specific target gene transcription independently to glucose metabolism. (Cho et al., 2006).

Sucrose transporter 2 (SUC2) has a central role in the phloem loading of sucrose and is essential for high performance sucrose transport from source to sink organs. A paper by Tong et al., revealed that SUC2 be regulated by another sugar, glucose by the HXK1-EIN3-SUC2 module (Tong et al., 2022). This module has been found to promote sucrose phloem loading, raising sucrose levels in the roots facilitating glucose signalling mediated root growth. Ethylene Insensitive 3 (EIN3) is a transcription factor involved in ethylene signalling

pathway in plants. EIN3 acts downstream of Arabidopsis hexokinase1 (HXK1), an enzyme that is involved in the first step of glycolysis, the metabolic pathway that converts glucose into energy. The transcription factor EIN3 binds directly to the SUC2 promoter, inhibiting SUC2 expression which suppresses root growth. This module therefore has been found to promote the phloem loading of sucrose in the source tissues, ultimately raising the levels of sucrose in the roots. This results in glucose signalling mediated root growth (Tong et al., 2022). When it comes to the *Beta vulgaris* SUC1 transporter is that sucrose negatively regulates the transport of sucrose and the steady state of the mRNA levels. This is in contrast to the increase in sucrose and glucose accumulation observed in *Arabidopsis.* (Vaughn et al., 2002) Excess glucose found in the source tissues trigger ethylene production which leads to an increase in EIN3 levels which consequently led to the inhibition of SUC2 activity delaying root growth (Tong et al., 2022).

To conclude, HXK has a multifaceted role contributing to the plant's growth and development. Hexokinase has the ability to form protein complexes that affect gene expression and that can promote root growth as shown in the evidence presented in this review. There is a possibility that it is able to form protein complexes with other molecules that are involved in sensing sugar levels over a long distance. Although HXK functions primarily intracellularly which might not necessarily seem the obvious choice to suggest it as a long-distance sugar signal however it is known that it can modulate specific target gene transcription which allows it to participate in complex regulatory networks that control the plant's growth and development. Also, its role in the regulation of sucrose transport, potentially, with more research, could reveal a signalling complex involving HXK that contribute to long-distance sugar signalling.

Target Of Rapamycin (TOR)

Another pathway of the sugar signalling network in plants that I wanted to explore was Target of Rapamycin (TOR). TOR's pivotal role as a master regulator in plant growth and its responsiveness to sugar could possibly make it part of long-distance sugar signalling as well (Kravchenko et al.,2015). Target of rapamycin (TOR) kinase is evolutionarily conserved in eukaryotic organisms and it's a key protein kinase that integrates information about nutrients, hormone levels, energy and transduces into growth and developmental decisions (Kravchenko et al.,2015). In plants, TOR-kinase forms a complex with Regulatory-Associated Protein of TOR (RAPTOR) and (Leucine Sensing Transporter 8 or GβL (LST8) called the TOR complex 1 (TORC1). RAPTOR is a multidomain protein that regulates the catalytic activity, stability as well as the substrate binding TORC1. LST8 is a WD40-domain protein stabilising the TOR kinase domain, serving as a scaffold for protein interaction (Burkart et al., 2021). This complex regulates metabolism and growth through the synthesis of proteins in a positive manner (Kravchenko et al., 2015). This complex is activated by sugars, auxins, light, ATP, inorganic nutrients and amino acids. (Pacheco et al. 2021; Liu and Xiong 2022; Artins and Caldana 2022) is inhibited by energy deprivation, starvation and stresses in plants. (Dobrenel et al., 2016a; González and Hall, 2017; Saxton and Sabatini, 2017; Xiong and Sheen, 2015).

A study that caught my eye was a study by Yuan et al., where as part of their investigation they wanted to reveal the role of PIN2 and how it is involved in the regulation of glucose-TOR of the auxin gradient. PIN2 is a protein that serves as transporter of auxin, mediating the transport of auxin shootward in the roots (Xu et al., 2005). When glucose was depleted or TOR was inhibited in the primary root, PIN2-GFP level was strongly diminished in the differentiation and the elongation zones while PIN2'S polarised location still maintained. Investigating the *pPIN2::PIn2-GFP* reporter line revealed that TOR glucose signalling affects PIN2 localisation and expression in the roots and regulates PIN2 degradation (Yuan et al., 2020). There is a direct interaction between TOR kinase and the central hydrophilic loop domain of PIN2, and TOR is a PIN2 stabiliser. This signalling pathway is important in order to be able to maintain a low auxin response region in the elongation zone, promoting cell expansion by regulating PIN2 expression. When the TOR signalling pathway is inhibited,

it leads to a decrease in polar PIN2 region and leads to the blockage of auxin transport from the elongation to the differentiation zone, resulting in high auxin accumulation as well as root expansion inhibition (Yuan et al., 2020). In this study it was revealed that PIN2 is a substrate of TOR, as TOR can phosphorylate and stabilise PIN2. TOR stabilises and phosphorylates PIN2, influencing the gradient distribution of PIN2 in the primary root of *Arabidopsis.* Glucose-TOR signalling is important for maintaining a low auxin response region in the elongation zone so that cell expansion is promoted by regulating the expression of PIN2. They proposed that when glucose-TOR signalling is inhibited the region where polarised PIN2 is located in the elongation zone decreases largely and so the transport of auxin from the elongation zone to the differentiation zone is blocked. This led to a high accumulation of auxin in the elongation zone and the inhibition of expansion in *Arabidopsis* root (Yuan et al., 2020).

Proposing TOR as a long-distance sugar signal seems quite unlikely as glucose isn't found in the phloem and TOR signalling as well as glucose functions intracellularly. However, the activity of TORC1 intracellularly, has systemic impacts affecting whole-plant growth and development and I do think that further investigations could possibly reveal its role in sugar signalling also in the context of long distance. A common theme with TOR is that it interacts with hormonal pathways which I discuss more in detail later in this review.

SNF1-related protein kinase 1 (SnRK1)

SNF1-related protein kinase 1 (SnRK1) is one of the well-established sugar signalling pathways and I set out to see if there is any evidence that this pathway also acts in long-distance. AtKIN10/AtKIN11/SnRK1 stands as a serine/threonine kinase, exhibiting significant sequence similarity to yeast SnF1 and mammalian AMPK (50 AMP-activated Protein Kinase). This kinase serves as a key energy signalling hub which regulates numerous proteins (Broeck et al., 2016; Simon et al., 2018). SnRK1's evolutionary conservation of its function is evident by looking at its structure, which is heterotrimeric, consisting of a catalytic α-subunit and two regulatory ß and γ subunits (Polge et al., 2007). This kinase has an essential role in the metabolic reprogramming, growth and development adjustments and responses to various abiotic and biotic stresses (Broeck et al., 2016). It controls the expression of over a thousand genes, including transcription factors and proteins that are involved in chromatin remodelling. It also exerts post-transcriptional regulation on key metabolic enzymes and specific transcription factors (Sugden et al., 1999; Harthill et al., 2006; Baena-Gonzalez et al., 2007, Coello et al., 2010; Hey et al., 2009). The expression profile that is regulated by SnRK1 correlates positively with genes that are regulated by sugar starvation (Baena Gonzalez et al., 2007). Research has revealed that the phosphorylation of a conserved threonine residue near the active site in the catalytic α-subunit is essential for regulating the activity and signalling of SnRK1. PP2C phosphatases dephosphorylate SnRK1a, possibly, reversing the activation loop which provides mechanisms for integrating environmental cues (Rodrigues et al., 2013). SnRKs are known to interact with ABA signalling, and the interplay of SnRK2/ABA and SnRK1 with PP2C phosphatases is demonstrated by both kinase types sharing common downstream targets such as various bZIP transcription factors (Hey et al., 2009). Furthermore, the potential involvement of microRNAs in SnRK1-mediated signal transduction is noteworthy (Confraria et al., 2013).

As I was exploring any evidence that could support the notion that SnRK1 could be involved in long-distance sugar signalling I stumbled upon a recent paper that has some very interesting findings about how SnRK1 in peaches plays a role in sucrose-mediated root growth though auxin signalling. This study was conducted on peaches and it revealed PpSnRK1 participates in the sucrose-mediated root growth through auxin signalling (Zhang et al., 2020). Lateral roots are important as they increase the surface area for absorption in the root system. There are several factors that affect the development of lateral roots. Examples include nutrients, plant hormones like auxin, biotic and abiotic stresses. Sugar found exogenously, in particular sucrose plays a vital role in the formation of lateral roots. Sucrose affects auxin transport and its distribution which

consequently influences hypocotyl elongation. Their study revealed that SnRK1 (SNF1/AMPK/SnRK1 protein kinase) is conserved and regulates glucose metabolism. When the roots were subjected to 5% sucrose treatment SnRK1 activity was upregulated. T6P inhibits the activity of SnRK1 and sucrose reverses this inhibitory effect of trehalose on SnRK1 and the growth of roots. In the study they overexpressed PpSnRK1 in *Arabidopsis* and they found that total root surface as well as number of lateral roots had increased. Also, the overexpression led to the upregulation of the genes involved in the auxin transport and synthesis. There is an interaction between PpSnRK1 and IAA12 as well as PIN-LIKE6 proteins in the auxin signalling pathway. Sucrose upregulates the expression of IAA12 and PIN6 genes, whereas trehalose inhibits their expression. Sucrose dismisses the inhibition that is induced by trehalose when they were applied together. SnRK1 is regulated by sucrose promoting the gene expression of auxin-related as well as interacting with auxin related proteins. SnRK1 acts as an intermediate signal connecting auxin and sucrose signals which regulate the growth of roots in peaches (Zhang et al., 2020).

SnRK1 controls the expression of thousands of genes and transcription factors as well as regulating post-transcription of key metabolic enzymes of specific transcription factors which makes me believe that it could possibly be also controlling the expression of genes and the transcription of genes that are involved in the sugar signalling over a long distance. SnRK1 like TOR appear to have a role in sugar signalling by interacting with different hormones like auxin, which I go into more detail below.
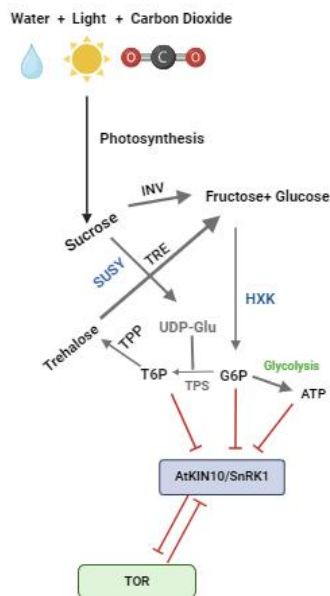
Figure 1. A simple schematic representation of sugar metabolism (green lettering) and signaling (blue lettering) pathways that have been discussed in this literature review. Glucose sensors like Hexokinase (HXK), Downstream of sugar perception are two energy sensors: Sucrose-non-fermentation-related protein kinase1 (AtKIN10/SnRK1) and Target of Rapamycin Kinase (TOR-kinase). Glucose 6-phosphate (G6P); invertase (INV), T6P synthase (TPS) ; trehalose 6-phosphate (T6P); trehalose 6P phosphatase (TPP); Trehalose 6P synthase (TPS). *Made with Biorender*.

Cross-talk Between Sugar Signalling and Plant Hormones

In the sugar signalling literature I reviewed there was a common theme of crosstalk between different signalling pathways, and growing evidence establishing the crosstalk of sugar signalling with plant hormones. I explored this idea to see if any of this crosstalk could potentially attribute to sugar signalling in the context of long distance. Sucrose, auxin and cytokinin have been identified to have strong links with sucrose signalling and sensing. They can function as both short and long-distance signalling molecules which suggests that they have a role in integrating growth and development for between shoots to roots. However, these interactions are not well-studied, even in *Arabidopsis*. There is a connection between the production of auxin and sucrose

(Le et al., 2010; Lilley et al., 2012; Sairanen et al., 2012) which makes it a good candidate for a long-distance signal as it promotes the production of lateral roots. Auxin (indole-3-3 acetic acid, IAA) is a plant hormone that has a crucial role in several growth and development processes (Olatunki et al., 2017; Majda et al.,2018; Tian et al., 2018).

Sugar contents fluctuate throughout the day, and these fluctuations are correlated with auxin levels (Sairanen et al. 20120), the circadian clocks gates (Covington et al., 2007) and the sensitivity to auxin treatment. The supplementation of sucrose induces YUCCA9 (Lilley et al., 2012; Covington et al. 2007) in the shoots but not in the roots which suggests that sugars might have an impact in the transportation of auxin as well as conjugation pathways. YUCCA9 belongs to the gene family of YUCCA (Cao et al., 2019). These genes are involved in the biosynthesis of auxin. The effect sucrose has on the promotion of growth is probable through the impact it has on auxin, as it can be partly imitated by adding auxin and can be blocked by adding polar auxin inhibitors. An example of the link between auxin signalling and sugar metabolism is shown in the down-regulation of the tomato auxin response repressor Auxin Response Factor 4 (SIARF4) which results in an increase in number of chloroplasts and starch and sugar content in the fruit (Sagar et al., 2013).

Another example showcasing the crosstalk between sugar and auxin is a study by Moore et al., involving IAA metabolism. They revealed that *Arabidopsis* hypocotyl explants of *hxk/gin2* were insensitive to auxin induction, which revealed the relationship between sugars and auxin (Moore et al., 2003). It was shown that mutants that were resistant to auxin are insensitive to high glucose concentrations (Moore et al., 2003). This interaction of auxin and sugars influence various plant processes including cell differentiation, hypocotyl, anther development (Wang et al., 2013; Eveland et al., 2011; Min et al., 2016; Li et al., 2016; Moreno-Ortega et al., 2014). The negative effect of auxin on sugar responsive genes was further confirmed by investigating *hookless* mutant (*hls1*) which was found to show resistance to both auxin and sugar responses (Ohto et al., 2006). In addition to that, transcriptomic experiments have shown glucose regulation of auxin-related genes and demonstrated the crosstalk of glucose and auxin which influences root architecture (Mishra et al., 2009; Eveland et al., 2011; Jain et al., 2007; MacGregor et al.,2008; Kircher et al., 2012). Transcription factors like S1bZIP11-related transcription factors were identified as negatively regulating auxin-mediated primary root growth, responding to changes in sugar levels and low energy through the activity of SnRK1 kinase (Weiste et al., 2017). The interplay in root growth between glucose and auxin involves the heterotrimeric G-protein, TOR-kinase signalling pathway and MED12/MED13 which are subunits of the MEDIATOR (multi-subunit protein complex that plays a crucial role in transcriptional regulation) complex. PIF (Phytochrome-Interacting Factors) transcription factors also have a role in connecting auxin accumulation and sugar signalling during the elongation of hypocotyl (Raya- González et al., 2017) which impacts the plant's development. In general, auxin and sugar have a crucial, entangled role in the plant growth and development working on an individual as well as a cooperative level.

Cytokinins are a class of hormones, essential for plant growth, senescence and stress tolerance (Albacete et al., 2014). A study on rice has revealed that when the cytokinin biosynthetic gene Isopentenyl Transferase (IPT) was overexpressed under a stress-induced promoter increasing the stress tolerance of rice under drought (Reguera et al., 2013). This is associated with an increase in sucrose content and maintained by the nitrate acquisition in the root system. Other evidence includes high levels of carbon dioxide in Arabidopsis seedlings, increase the growth of the roots especially under abiotic stress conditions, which is associated with changes in the levels of cytokinin, glucose, starch, auxin and sucrose (Hachiya et al. 2014). A study on tomato gave evidence of a link between cytokinin biosynthesis and storage-organ formation (Eviatar et al., 2013). They revealed that the overexpression of the LONELY GUY 1 (LOG1), a gene involved in cytokinin biosynthesis, induced the formation of tuber-like organs from the axillary meristems. There is an interaction of sugars and cytokinins during growth and development which affect developmental pathway and stress responses. Transcript profiling of *Arabidopsis* seedlings showed that after the seeds were treated with cytokinin and glucose they showed agonists and antagonistic effects on gene expression, particularly glucose

affecting genes that are involved in the signalling and metabolism of cytokinin (Kushwah et al., 2014). When the plant suffers from cytokinin deficiency, which is caused by the constituent overexpression of cytokinin oxidase (CKX) genes it leads to drastic changes in the root and shoot growth which involves the alterations in the cell cycle, carbohydrate distribution, photosynthetic activity and source/sink relations (Werner et al., 2008).

There is overwhelming evidence supporting the crosstalk between sugar signalling pathways and hormonal pathways, so, it would make sense that these interactions act over long distances as well. Acting as both short and long-distance signalling molecules, auxin and also cytokinins to some extent, exhibit connections with growth and development, particularly between shoots and roots. Plant hormones regulate the plant's growth and development on a systemic level, they are mobile so they can travel over distance, the connection of sugars and hormones contributing to root growth and the correlation with changes in sugar levels make plant hormones a big part of the sugar signalling network. The evidence presented makes me believe that a substantial portion of long-distance sugar signalling likely relies on the interplay between hormonal and sugar signalling pathways.

Abiotic stress and its effect on long distance sugar signalling

Sugar signalling pathways have the purpose of adjusting and sensing changes in sugar levels in response to changes in the plant's environment. Therefore, it only felt appropriate to also explore the effect different abiotic stresses have on sugar signalling and potentially identifying any evidence that could be linked to long-distance sugar signalling as many of these stresses directly affect the root system of the plant. In order for roots to grow, they depend on sucrose that is derived from the shoot of the plant. When the plant is under stress the availability of sucrose becomes limited due to insufficient photosynthesis and accumulation of sugar in the shoot (Thalmann et al., 2017). Under abiotic stress, sucrose allocation to the root's changes. Sucrose transport in *Arabidopsis* takes place in the phloem and is regulated mostly by two sugar transporters Sugar Will Eventually be Exported Transporters 11 and 12 (SWEET11 and 12) (Chen et al., 2012). These two sugar transporters channel sucrose from the parenchyma cells which are located in the phloem, to the apoplast. SUC2 transports apoplastic sucrose into the companion cells in the phloem (Chen et al., 2012; Gottwald et al. 2000; Xu et al., 2020). Once the sucrose reaches the roots, sucrose can be unloaded apoplastically and symplastically. ELONGATED HYPOCOTYL5 (HY5) is a bZIP-type transcription factor that regulates various biological and physiological processes like root growth, nutrient acquisition, photomorphogenesis and responses to abiotic stress. HY5 promotes the expression of SWEET11 and SWEET12 in order to enhance sucrose loading in the phloem in the light (Chen et al., 2016). This is an example of integration of light cues from shoot-to-root by a mobile transcription factor HY5 to sucrose distribution, but still, HY5 does not fully act as a long-distance sugar signal. When the plant is exposed to light or is under nutrient-deficient conditions it has increased levels of sucrose in the phloem as it wants to invest in root growth. Sucrose and glucose that are derived from the shoot are crucial for root growth, and HEXOKINASE 1, ARABIDOSIS PROTEIN KINASEs, and TOR are vital for root growth and perception of sucrose and glucose (Chen et al., 2016). This requires communication between the shoot and the roots to coordinate developmental and physiological processes. Sucrose is not a rapid signal as it depends on photosynthesis and its involvement in several molecular processes (Tognetti et al., 2013). HY5 gets transmitted form the shoot to the root through the phloem and has a role in promoting root growth as well as nitrate uptake by the activation of NRT2.1 by light (Chen et al., 2016). It was revealed that light induces HY5 in the shoot and that in turn upregulates the expression of PHYTOENE SYNTHASE, TREHALOSE-6-PHOSPHATE SYNTHASE, SWEET11, and SWEET12, involved in carbon fixation as well as the translocation of photosynthates from shoot to root which suggests that HY5 has a role as a mobile signal that coordinates growth of the shoot and root in a light dependent manner (Chen et al., 2016).

Under salt and drought stress, ABA-induced transcription factors Abscisic Acid-Responsive Element-Binding Protein 3 (AREB3) and Abscisic Acid-Insensitive 5 (ABI5) bind to the promoter of *AtSUC1* inhibiting its expression through ABA signalling (Hoth et al., 2010). As salt and drought stress lead to limited carbon status, so this process affects the sucrose transport to the sinks via ABA. Salt stress induces Basic Leucine Zipper 1 (bZIP1) and Basic Leucine Zipper 53 (bZIP53) expression downstream of SnRK1, which is crucial for the metabolic reprogramming of primary carbohydrates in *Arabidopsis* roots (Hartmann et al., 2015). Low energy signalling of SnRK1 activates Basic Leucine Zipper 11 (bZIP11) which directly activates INDOLE-3-ACETIC ACID INDUCIBLE 3/SHORT HYPOCOTYL 2 (IAA3/SHY2) SHY2/IAA3 transcription in the root which leads to a decrease in auxin transport as well as limited primary root growth. In the lateral roots, WUSCHEL RELATED HOMEOBOX 7 (WOX7) suppresses the initiation of lateral roots in a sucrose-dependent manner through the repression of CYCD6;1. Root primordia initiation is regulated by WOX7, working downstream of LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16) and WOX11/12 in an auxin-dependent manner (Hu et al., 2016). To summarise, SnRK1 signalling and sucrose originating from the shoot affect root development in response to environmental cues. Under water deficit conditions, enhanced carbon export from the shoot to the root supports deeper rooting. A possible scenario is SnRK1–TOR energy signalling pathway and its interaction with auxin and ABA signalling contributes to altered root growth under sugar-limited conditions (Margalha et al., 2019).

In light of the evidence presented, the dependency of root growth on sucrose derived from the shoot, the alterations in sucrose transport and regulation under abiotic stress, and the crosstalk between hormonal pathways, particularly involving SnRK1, TOR, and auxin signalling, strongly suggest that these work in combination to communicate sugar status long-distance in plants. Further research on different stresses could help reveal more details about long-distance sugar signalling and the connections between these signalling pathways.
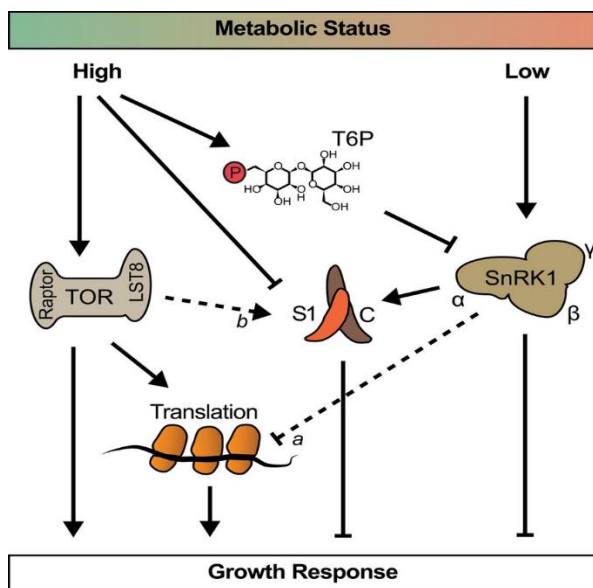
Figure 2. Sugar signalling pathways play a crucial role in regulating the plant's growth and development. Nutrient stress activates SnRK1 which leads to the inhibition of growth. SnRK1 target genes are governed by C/S1-Bzip transcription factors. Metabolic status is heightened by sucrose and this is correlated with T6P levels in plants. T6P inhibits the activity of SnRK1, while TOR kinase stimulates growth and translation processes. Sucrose has inhibitory effects on translating S1-group bZIP mRNAs. *Adapted from: Lastdrager et al., 2014.*

Conclusion

In this review I set out to review the well-established sugar signalling pathways and their crosstalk with other signalling pathways hoping for evidence that could point to the existence of a long-distance sugar signal or their involvement in long-distance sugar signalling. There was no direct evidence of a long-distance sugar signal in plants, however, by closely analysing literature involving these established sugar pathways I started to see the potential they have being part of long-distance sugar signalling. These signalling pathways and their interactions are so complex, that studying them over a long distance can be very complicated. With advancements in technology and interdisciplinary approaches, light can be shed on long-distance sugar signalling. Sugar signalling is fundamental for the plant's growth and development and having a better understanding of long-distance sugar signalling is essential for unravelling the coordination between the different parts of the plants, specifically shoots and roots as well as the integration of developmental and metabolic processes. Also, it will shed more light on the role of sugars as signals. Plants have to face everchanging environmental conditions, therefore, having a deep understanding of long-distance signalling will help with optimising plant health and their survival strategies.

References

1. Abelenda, J.A. et al. (2019) Source-sink regulation is mediated by interaction of an FT homolog with a SWEET protein in potato. Curr. Biol. 29, 1178–1186.

2. Agusti, J. and Blazquez, M.A. (2020) Plant vascular development: mechanisms and environmental regulation. Cell. Mol. Life Sci. 77, 3711–3728.

3. AK, Hofmann MG, Römer U, Köckenberger W, Elling L, Roitsch T. (2002) Metabolizable and non-metabolizable sugars activate different signal transduction pathways in tomato. Plant Physiol. Apr;128(4):1480-9. doi: 10.1104/pp.010771. PMID: 11950996; PMCID: PMC154275.

4. Albacete AA, Martinez-Andujar C, Perez-Alfocea F (2014): Hormonal and metabolic regulation of source-sink relations under salinity and drought: from plant survival to crop yield stability. Biotechnol Adv, 32:12-30.

5. Baena-González, E.; Rolland, F.; Thevelein, J.M.; Sheen, J.(2007) A central integrator of transcription networks in plant stress and energy signalling. Nature, 448, 938–942.

6. Barrada A, Montané MH, Robaglia C, Menand B. (2015) Spatial Regulation of Root Growth: Placing the Plant TOR Pathway in a Developmental Perspective. Int J Mol Sci. 2015 Aug 19;16(8):19671-97. doi: 10.3390/ijms160819671. PMID: 26295391; PMCID: PMC4581319.

7. Beveridge, C.A.; Lunn, J.E. (2017) Trehalose 6-phosphate is involved in triggering axillary bud outgrowth in garden pea (Pisum sativum L.). Plant J. 2017, 92, 611–623.

8. Booker, K.S.; Schwarz, J.; Garrett, M.B.; Jones, A.M. Glucose attenuation of auxin-mediated bimodality in lateral root formation is partly coupled by the heterotrimeric G protein complex. PLoS ONE 2010, 5, e12833. Int. J. Mol. Sci. 2018, 19, 2506 30 of 42

9. Broeckx, T.; Hulsmans, S.; Rolland, F. (2016) The plant energy sensor: Evolutionary conservation and divergence of SnRK1 structure, regulation, and function. J. Exp. Bot. 2016, 67, 6215–6252.

10. Burkart GM, Brandizzi F. A Tour of TOR Complex Signaling in Plants. Trends Biochem Sci. 2021 May;46(5):417-428. doi: 10.1016/j.tibs.2020.11.004. Epub 2020 Dec 9. PMID: 33309324; PMCID: PMC8052268.

11. Burko, Y. et al. (2020) Local HY5 activity mediates hypocotylgrowth and shoot-to-root communication. Plant Commun. 1,10007852. Chen, W.W. et al. A mobile ELF4 delivers circadian temperature information from shoots to roots. Nat Plants 6, 416–426.

12. Cabib, E.; Leloir, L.F. The biosynthesis of trehalose phosphate. J. Biol. Chem. 1958, 231, 259–275. [PubMed]

13. Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, et al. (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science. ; 335: 207–211. pmid:22157085

14. Chen, W.W. et al. (2020) A mobile ELF4 delivers circadian temperature information from shoots to roots. Nat Plants 6, 416–426

15. Chen, X. et al. (2016) Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. Curr. Biol. 26, 640–646

16. Chiou, T. J., & Bush, D. R. (1998). Sucrose is a signal molecule in assimilate partitioning. Proceedings of the National Academy of Sciences, 95(8), 4784–4788. https://doi.org/10.1073/pnas.95.8.4784

17. Cho, Y.-H., Yoo, S.-D., & Sheen, J. (2006). Regulatory Functions of Nuclear Hexokinase1 Complex in Glucose Signaling. Cell, 127(3), 579–589. https://doi.org/10.1016/j.cell.2006.09.028

18. Coello, P.; Hey, S.J.; Halford, N.G. (2010) The sucrose non-fermenting-1-related (SnRK) family of protein kinases: Potential for manipulation to improve stress tolerance and increase yield. J. Exp. Bot. 62, 883–893.

19. Confraria, A.; Martinho, C.S.D.S.; Elias, A.; Rubio-Somoza, I.; Baena-González, E. (2013) miRNAs mediate SnRK1-dependent energy signaling in Arabidopsis. Front. Plant Sci. 4, 197.

20. Covington MF, Harmer SL (2007): The circadian clock regulates auxin signaling and responses in Arabidopsis. PLoS Biol 2007, 5:e222

21. Dobrenel, T.; Marchive, C.; Sormani, R.; Moreau, M.; Mozzo, M.; Montané, M.H.; Menand, B.; Robaglia, C.; Meyer, C. (2011) Regulation of plant growth and metabolism by the TOR kinase. Biochem. Soc. Trans. , 39,477–481.

22. Dornbusch T, Michaud O, Xenarios I, Fankhauser C (2014): Differentially phased leaf growth and movements in Arabidopsis depend on coordinated circadian and light regulation. Plant Cell.

23. Durand, M. et al. (2016) Water deficit enhances C export to the roots in Arabidopsis thaliana plants with contribution of sucrose transporters in both shoot and roots. Plant Physiol. 170, 1460–1479.

24. Durand, M. et al. (2018) Carbon source-sink relationship in Arabidopsis thaliana: the role of sucrose transporters. Planta 247, 587–611

25. Eveland, A.L.; Jackson, D.P. (2011) Sugars, signalling, and plant development. J. Exp. Bot. 63, 3367–3377.

26. Eviatar-Ribak T, Shalit-Kaneh A, Chappell-Maor L, Amsellem Z, Eshed Y, Lifschitz E (2013): A cytokinin-activating enzyme promotes tuber formation in tomato. Curr Biol, 23:1057-1064.

27. Fernie, A. R., & Willmitzer, L. (2001). Molecular and Biochemical Triggers of Potato Tuber Development. Plant Physiology, 127(4), 1459–1465. https://doi.org/10.1104/pp.010764

28. Fichtner F, Olas JJ, Feil R, Watanabe M, Krause U, Hoefgen R, Stitt M, Lunn JE. Functional Features of TREHALOSE-6-PHOSPHATE SYNTHASE1, an Essential Enzyme in Arabidopsis. Plant Cell. 2020

Jun;32(6):1949-1972. doi: 10.1105/tpc.19.00837. Epub 2020 Apr 10. PMID: 32276986; PMCID: PMC7268806.

29. Figueroa, C.M.; Lunn, J.E. (2016) A tale of two sugars: Trehalose 6-phosphate and sucrose. Plant Physiol. 172, 7–27. [CrossRef] [PubMed] Int. J. Mol. Sci. 2018, 19, 2506 21 of 42.

30. Gonzali, S.; Novi, G.; Loreti, E.; Paolicchi, F.; Poggi, A.; Alpi, A.; Perata, P. (2005) A turanose-insensitive mutant suggests a role for WOX5 in auxin homeostasis in Arabidopsis thaliana. Plant J. , 44, 633–645.

31. Gottwald JR, Krysan PJ, Young JC, Evert RF, Sussman MR.(2000) Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proceedings of the National Academy of Sciences.; 97: 13979–13984. pmid:11087840

32. Granot, D., David-Schwartz, R., & Kelly, G. (2013). Hexose Kinases and Their Role in Sugar-Sensing and Plant Development. Frontiers in Plant Science, 4. https://doi.org/10.3389/fpls.2013.00044

33. Hachiya T, Sugiura D, Kojima M, Sato S, Yanagisawa S, Sakakibara H, Terashima I, Noguchi K (2014): High CO2 triggers preferential root growth of Arabidopsis thaliana via two distinct systems under low pH and low N stresses. Plant Cell Physiol, 55:269-280.

34. Hammond, J. P., & White, P. J. (2011). Sugar Signaling in Root Responses to Low Phosphorus Availability. Plant Physiology, 156(3), 1033–1040. https://doi.org/10.1104/pp.111.175380

35. Harthill, J.E.; Meek, S.E.; Morrice, N.; Peggie, M.W.; Borch, J.; Wong, B.H.; MacKintosh, C. (2006) Phosphorylation and 14-3-3 binding of Arabidopsis trehalose-phosphate synthase 5 in response to 2-deoxyglucose. Plant J., 47, 211–223.

36. Hartmann, L. et al. (2015) Crosstalk between two bZIP signaling pathways orchestrates salt-induced metabolic reprogramming in Arabidopsis roots. Plant Cell 27, 2244–2260

37. Hartmann, L.; Pedrotti, L.; Weiste, C.; Fekete, A.; Schierstaedt, J.; Göttler, J.; Kempa, S.; Krischke, M.; Dietrich, K.; Mueller, M.J.; et al.(2015) Crosstalk between two bZIP signaling pathways orchestrates salt-induced metabolic reprogramming in Arabidopsis roots. Plant Cell.

38. Hey, S.J.; Byrne, E.; Halford, N.G. (2009) The interface between metabolic and stress signalling. Ann. Bot., 105, 197–203.

39. Hoth, S. et al. (2010) An ABA-responsive element in the AtSUC1 promoter is involved in the regulation of AtSUC1 expression. Planta 232, 911–923.

40. Hu, X. and Xu, L. (2016) Transcription factors WOX11/12 directly activate WOX5/7 to promote root primordia initiation and organogenesis. Plant Physiol. 172, 2363–2373.

41. J. Xu, B. Scheres (2005), Dissection of Arabidopsis ADP-RIBOSYLATION FACTOR 1 function in epidermal cell polarity. Plant Cell 17, 525–536).

42. Jain, A.; Poling, M.D.; Karthikeyan, A.S.; Blakeslee, J.J.; Peer, W.A.; Titapiwatanakun, B.; Murphy, A.S.; Raghothama, K.G. (2007) Differential effects of sucrose and auxin on localized phosphate deficiency-induced modulation of different traits of root system architecture in Arabidopsis. Plant Physiol., 144, 232–247.

43. Jang J. C., Sheen J. (1997). Sugar sensing in higher plants. Trends Plant Sci. 2, 208–214 10.1016/S1360-1385(97)89545-3.

44. Kelly, Gilor, et al. "The Pitfalls of Transgenic Selection and New Roles of AtHXK1: A High Level of AtHXK1 Expression Uncouples Hexokinase1-Dependent Sugar Signaling from Exogenous Sugar." Plant

Physiology, vol. 159, no. 1, 2012, pp. 47–51. JSTOR, http://www.jstor.org/stable/41496244. Accessed 10 Mar. 2024.

45. Kircher, S. and Schopfer, P. (2012) Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 109, 11217–11221

46. Kircher, S.; Schopfer, P. (2012) Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in Arabidopsis. Proc. Natl. Acad. Sci. USA 2012, 109, 11217–11221.

47. Kong, D. et al. (2016) The WUSCHEL related homeobox protein WOX7 regulates the sugar response of lateral root development in Arabidopsis thaliana. Mol. Plant 9, 261–270

48. Kravchenko, A.; Citerne, S.; Jéhanno, I.; Bersimbaev, R.I.; Veit, B.; Meyer, C.; Leprince, A.S. (2015) Mutations in the Arabidopsis Lst8 and Raptor genes encoding partners of the TOR complex, or inhibition of TOR activity decrease abscisic acid (ABA) synthesis. Biochem. Biophys. Res. Commun., 467, 992–997.

49. Kravchenko, A.; Citerne, S.; Jéhanno, I.; Bersimbaev, R.I.; Veit, B.; Meyer, C.; Leprince, A.S. (2015) Mutations in the Arabidopsis Lst8 and Raptor genes encoding partners of the TOR complex, or inhibition of TOR activity decrease abscisic acid (ABA) synthesis. Biochem. Biophys. Res. Commun. 467, 992–997.

50. Kushwah S, Laxmi A: The interaction between glucose and cytokinin signal transduction pathway in Arabidopsis thaliana. Plant Cell Environ 2014, 37:235-253.

51. Lastdrager, J., Hanson, J., & Smeekens, S. (2014). Sugar signals and the control of plant growth and development. Journal of Experimental Botany, 65(3), 799–807. https://doi.org/10.1093/jxb/ert474

52. LeClere, S.; Schmelz, E.A.; Chourey, P.S. Sugar levels regulate tryptophan-dependent auxin biosynthesis in developing maize kernels. Plant Physiol. 2010, 153, 306–318.

53. Lemoine, R.; Porcheron, B.; et al. Sucrose is an early modulator of the key hormonal mechanisms controlling bud outgrowth in Rosa hybrida. J. Exp. Bot. 2015, 66, 2569–2582.

54. Leyman, B.; Van Dijck, P.; Thevelein, J.M (2001) An unexpected plethora of trehalose biosynthesis genes in Arabidopsis thaliana. Trends Plant Sci.6, 510–513.

55. Li X, Cai W, Liu Y, Li H, Fu L, Liu Z, Xu L, Liu H, Xu T, Xiong Y. (2017) Differential TOR activation and cell proliferation in Arabidopsis root and shoot apexes. Proc Natl Acad Sci U S A., Mar 7;114(10):2765-2770. doi: 10.1073/pnas.1618782114. Epub 2017 Feb 21. PMID: 28223530; PMCID: PMC5347562.

56. Li, G.; Ma, J.; Tan, M.; Mao, J.; An, N.; Sha, G.; Zhang, D.; Zhao, C.; Han, M. (2016) Transcriptome analysis reveals the effects of sugar metabolism and auxin and cytokinin signaling pathways on root growth and development of grafted apple. BMC Genom., 17, 150.

57. Lilley JL, Gee CW, Sairanen I, Ljung K, Nemhauser JL (2012): An endogenous carbon-sensing pathway triggers increased auxin flux and hypocotyl elongation. Plant Physiol , 160:2261-2270.

58. Lin, X.Y.; Ye, Y.Q.; Fan, S.K.; Jin, C.W.; Zheng, S.J. (2016) Increased sucrose accumulation regulates iron-deficiency responses by promoting auxin signaling in Arabidopsis plants. Plant Physiol., 170, 907–920.

59. Liu, Y.; Bassham, D.C. (2010) TOR is a negative regulator of autophagy in Arabidopsis thaliana. PLoS ONE, 5,e11883.

60. Ljung, K., Nemhauser, J. L., & Perata, P. (2015). New mechanistic links between sugar and hormone signalling networks. Current Opinion in Plant Biology, 25, 130–137. https://doi.org/10.1016/j.pbi.2015.05.022

61. Long, J.C.; Zhao, W.; Rashotte, A.M.; Muday, G.K.; Huber, S.C. (2002) Gravity-stimulated changes in auxin and invertase gene expression in maize pulvinal cells. Plant Physiol., 128, 591–602.

62. Loreti E, Alpi A, Perata P (2000) Glucose and disaccharide-sensing mechanisms modulate the expression of α-amylase in barley embryos. Plant Physiol 123: 939–948

63. MacGregor, D.R.; Deak, K.I.; Ingram, P.A.; Malamy, J.E. (2008) Root system architecture in Arabidopsis grown in culture is regulated by sucrose uptake in the aerial tissues. Plant Cell, 20, 2643–2660.

64. Mair, A.; Pedrotti, L.; Wurzinger, B.; Anrather, D.; Simeunovic, A.; Weiste, C.; Valerio, C.; Dietrich, K.; Kirchler, T.; Nagele, T.; et al. (2015) SnRK1-triggered switch of bZIP63 dimerization mediates the low-energy response in plants. eLife, 4, e05828.

65. Majda, M.; Robert, S. (2018) The role of auxin in cell wall expansion. Int. J. Mol. Sci. 19, 951.

66. Margalha, L. et al. (2019) SnRK1 and TOR: modulating growthdefense trade-offs in plant stress responses. J. Exp. Bot. 70, 2261–2274

67. M'Batchi, B., & Delrot, S. (1984). Parachloromercuribenzenesulfonic Acid. Plant Physiology, 75(1), 154–160. https://doi.org/10.1104/pp.75.1.154

68. Menand, B.; Desnos, T.; Nussaume, L.; Berger, F.; Bouchez, D.; Meyer, C.; Robaglia, C. (2002) Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. Proc. Natl. Acad. Sci. USA, 99, 6422–6427.

69. Michael TP, Breton G, Hazen SP, Priest H, Mockler TC, Kay SA, Chory J (2008): A morning-specific phytohormone gene expression program underlying rhythmic plant growth. PLoS Biol, 6:e225.

70. Milne, R.J. et al. (2018) Mechanisms of phloem unloading: shaped by cellular pathways, their conductances and sink function. Curr. Opin. Plant Biol. 43, 8–15

71. Min, L.; Li, Y.; Hu, Q.; Zhu, L.; Gao, W.; Wu, Y.; Ding, Y.; Liu, S.; Yang, X.; Zhang, X.; et al (2014). Sugar and auxin signaling pathways respond to high temperature stress during anther development as revealed by transcript profiling analysis in cotton. Plant Physiol.

72. Mishra, B.S.; Singh, M.; Aggrawal, P.; Laxmi, A. (2009) Glucose and auxin signaling interaction in controlling Arabidopsis thaliana seedlings root growth and development. PLoS ONE, 4, e4502.

73. Moore B., Zhou L., Rolland F., Hall Q., Cheng W. H., Liu Y. X., et al. (2003). Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300, 332–336 10.1126/science.1081418.

74. Moreno-Ortega, B.; Chandezon, E.; Fort, G.; Guédon, Y.; Muller, B.L. (2014) Why is lateral root growth so variable? A framework to analyze growth variability among lateral roots and the possible roles of auxin and carbon. In Proceedings of the 7th International Symposium on Root Development, Weimar, Germany, 15–19

75. O'Hara LE, Paul MJ, Wingler A. (2012). How do sugars regulate plant growth and development? New insight into the role of trehalose-6- phosphate. Molecular Plant 6, 261–274

76. Ohto, M.A.; Hayashi, S.; Sawa, S.; Hashimoto-Ohta, A.; Nakamura, K.(2006) Involvement of HLS1 in sugar and auxin signaling in Arabidopsis leaves. Plant Cell Physiol., 47, 1603–1611.

77. Olatunji, D.; Geelen, D.; Verstraeten, I. (2017)Control of endogenous auxin levels in plant root development. Int. J.Mol. Sci., 18, 2587.

78. Paparelli E, Parlanti S, Gonzali S, Novi G, Mariotti L, Ceccarelli N, van Dongen JT, Kolling K, Zeeman SC, Perata P (2013): Nighttime sugar starvation orchestrates gibberellin biosynthesis and plant growth in Arabidopsis. Plant Cell, 25:3760-3769

79. Paul, M.J.; Primavesi, L.F.; Jhurreea, D.; Zhang, Y. (2008), Trehalose metabolism and signaling. Annu. Rev. Plant Biol.m 59, 417–441.

80. Pereyra, C., Parola, R., Lando, A. P., Rodriguez, M., & Martínez-Noël, G. M. A. (2023). High Sugar Concentration Inhibits TOR Signaling Pathway in Arabidopsis thaliana. Journal of Plant Growth Regulation. https://doi.org/10.1007/s00344-022-10894-w

81. Polge, C.; Thomas, M. (2007). SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control? Trends Plant Sci.12, 20–28.

82. Ramachandran, P. et al. (2018) Continuous root xylem formation and vascular acclimation to water deficit involves endodermal ABA signalling via miR165. Development 145, dev159202.

83. Raya-González, J.; López-Bucio, J.S.; Prado-Rodríguez, J.C.; Ruiz-Herrera, L.F.; Guevara-García, Á.A.; López-Bucio, J. (2017) The MEDIATOR genes MED12 and MED13 control Arabidopsis root system configuration influencing sugar and auxin responses. Plant Mol. Biol., 95, 141–156.

84. Reguera M, Peleg Z, Abdel-Tawab YM, Tumimbang EB, Delatorre CA, Blumwald E (2013): Stress-induced cytokinin synthesis increases drought tolerance through the coordinated regulation of carbon and nitrogen assimilation in rice. Plant Physiol 2013, 163:1609-1622.

85. Ren, M.; Venglat, P.; Qiu, S.; Feng, L.; Cao, Y.; Wang, E.; Xiang, D.; Wang, J.; Alexander, D.; Chalivendra, S.; et al. (2012) Target of rapamycin signaling regulates metabolism, growth, and life span in Arabidopsis. Plant Cell 2012, 24, 4850–4874.

86. Riesmeier JW, Willmitzer L, Frommer WB. (1994) Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning. EMBO J., Jan 1;13(1):1-7. doi: 10.1002/j.1460-2075. 1994.tb06229.x. PMID: 8306952; PMCID: PMC394773.

87. Robaglia, C.; Thomas, M.; Meyer, C. (2012) Sensing nutrient and energy status by SnRK1 and TOR kinases. Curr.Opin. Plant Biol. 15, 301–307.

88. Rodrigues, A.; Adamo, M.; Crozet, P.; Margalha, L.; Confraria, A.; Martinho, C.; Elias, A.; Rabissi, A.; Lumbreras, V.; González-Guzmán, M.; et al (2013) ABI1 and PP2CA phosphatases are negative regulators of Snf1-related protein kinase1 signaling in Arabidopsis. Plant Cell.

89. Rolland F, Moore B, Sheen J. Sugar sensing and signaling in plants. Plant Cell.(2002);14 Suppl(Suppl):S185-205. doi: 10.1105/tpc.010455. PMID: 12045277; PMCID: PMC151255.

90. Ryabova, L.A. et al. (2019) Target of rapamycin kinase: central regulatory hub for plant growth and metabolism. J. Exp. Bot. 70, 2211–2216.

91. Sagar M, Chervin C, Roustant JP, Bouzayen M, Zouine M (2013): Underexpression of the auxin response factor Sl-ARF4 improves postharvest behavior of tomato fruits. Plant Signal Behav 2:8 http://dx.doi.org/10.4161/psb25647.

92. Sairanen I, Novak O, Pencik A, Ikeda Y, Jones B, Sandberg G, Ljung K (2012): Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in Arabidopsis. Plant Cell, 24:4907-4916.

93. Sakr S, Wang M, Dédaldéchamp F, Perez-Garcia MD, Ogé L, Hamama L, Atanassova R. (2018) The Sugar-Signaling Hub: Overview of Regulators and Interaction with the Hormonal and Metabolic Network. Int J Mol Sci.Aug 24;19(9):2506. doi: 10.3390/ijms19092506. PMID: 30149541; PMCID: PMC6165531.

94. Salem MA, Li Y, Bajdzienko K, Fisahn J, Watanabe M, Hoefgen R, Schöttler MA, Giavalisco P. (2018) RAPTOR Controls Developmental Growth Transitions by Altering the Hormonal and Metabolic Balance. Plant Physiol. Jun;177(2):565-593. doi: 10.1104/pp.17.01711. Epub 2018 Apr 23. PMID: 29686055; PMCID: PMC6001337.

95. Schluepmann H, Berke L, Sanchez-Perez GF. (2012). Metabolism control over growth: a case for trehalose-6-phosphate in plants. Journal of Experimental Botany 63, 3379–3390.

96. Simon, N.M.; Sawkins, E.; Dodd, A.N. (2018) Involvement of the SnRK1 subunit KIN10 in sucrose-induced hypocotyl elongation. Plant Signal. Behav.

97. Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. Curr Opin Plant Biol 13: 274–27

98. Srivastava AC, Ganesan S, Ismail IO, Ayre BG. (2008) Functional characterization of the Arabidopsis AtSUC2 Sucrose/H+ symporter by tissue-specific complementation reveals an essential role in phloem loading but not in long-distance transport. Plant Physiol. Sep;148(1):200-11. doi: 10.1104/pp.108.124776. Epub 2008 Jul 23. PMID: 18650401; PMCID: PMC2528097..

99. Stadler R, Sauer N. (2015) The Arabidopsis thaliana AtSUC2 Gene is Specifically Expressed in Companion Cells. Plant Biology.; 109(4): 299–306.

100. Stewart, J.L.; Maloof, J.N.; Nemhauser, J.L. (2018) PIF genes mediate the effect of sucrose on seedling growth dynamics. PLoS ONE 2011, 6, e19894. Int. J. Mol. Sci., 19, 2506 29 of 42

101. Stitt M, Zeeman SC 9(2012): Starch turnover: pathways, regulation and role in growth. Curr Opin Plant Biol, 15:282-292.

102. Sugden, C.; Crawford, R.M.; Halford, N.G.; Hardie, D.G. (1999) Regulation of spinach SNF1-related (SnRK1) kinases by protein kinases and phosphatases is associated with phosphorylation of the T loop and is regulated by 50-AMP. Plant J. 1999, 19, 433–439.

103. Teng, S.; Keurentjes, J.; Bentsink, L.; Koornneef, M.; Smeekens, S. (2005) Sucrose-specific induction of anthocyanin biosynthesis in Arabidopsis requires the MYB75/PAP1 gene. Plant Physiol.139, 1840–1852.

104. Thalmann, M. and Santelia, D. (2017) Starch as a determinant of plant fitness under abiotic stress. New Phytol. 214, 943–951.

105. Thom, M., & Maretzki, A. (1992). Evidence for Direct Uptake of Sucrose by Sugarcane Stalk Tissue. Journal of Plant Physiology, 139(5), 555–559. https://doi.org/10.1016/s0176-1617(11)80369-x

106. Tian, H.; Lv, B.; Ding, T.; Bai, M.; Ding, Z. (2018) Auxin-BR interaction regulates plant growth and development. Front. Plant Sci. 8, 2256.

107. Tong, C., Li, C., Cao, X.-Y., Sun, X.-D., Bao, Q.-X., Mu, X.-R., Liu, C.-Y., Loake, G. J., Chen, H.-h., & Meng, L.-S. (2022). Long-distance transport of sucrose in source leaves promotes sink root growth by the EIN3-SUC2 module. PLOS Genetics, 18(9), e1010424. https://doi.org/10.1371/journal.pgen.1010424

108.	Turgeon R, Wolf S (2009) Phloem transport: cellular pathways and molecular trafficking. Annu Rev Plant Biol 60: 207–221

109.	van Gelderen, K. et al. (2018) Far-red light detection in the shoot regulates lateral root development through the HY5 transcription factor. Plant Cell 30, 101–116

110.	Vaughn MW, Harrington GN, Bush DR. (2002) Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem. Proceedings of the National Academy of Sciences. 99(16): 10876–10880. pmid:12149483

111.	Wang, L.; Ruan, Y.L. Regulation of cell division and expansion by sugar and auxin signaling(2013) . Front. Plant Sci. 4, 163.

112.	Wang, M., Zang, L., Jiao, F., Perez-Garcia, M.-D., Ogé, L., Hamama, L., Le Gourrierec, J., Sakr, S., & Chen, J. (2020). Sugar Signaling and Post-transcriptional Regulation in Plants: An Overlooked or an Emerging Topic? Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.578096

113.	Weiste, C. et al. (2017) The Arabidopsis bZIP11 transcription factor links low-energy signalling to auxin-mediated control of primary root growth. PLoS Genet. 13, e1006607

114.	Weiste, C.; Dröge-Laser, W. (2014) The Arabidopsis transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetylation machinery. Nat. Commun. 5, 3883.

115.	Werner T, Holst K, Pors Y, Guivarc'h A, Mustroph A, Chriqui D, Grimm B, Schmulling T (2008): Cytokinin deficiency causes distinct changes of sink and source parameters in tobacco shoots and roots. J Exp Bot, 59:2659-2672.

116.	Wiese A, Christ MM, Virnich O, Schurr U, Walter A (2007): Spatiotemporal leaf growth patterns of Arabidopsis thaliana and evidence for sugar control of the diel leaf growth cycle. New Phytol, 174:752-761.

117.	Wind J, Smeekens S, Hanson J (2010) Sucrose: metabolite and signaling molecule. Phytochemistry 71: 1610–1614

118.	Xiong, Y.; McCormack, M.; Li, L.; Hall, Q.; Xiang, C.; Sheen, J. (2013) Glucose–TOR signalling reprograms the transcriptome and activates meristems. Nature, 496, 181–186.

119.	Xiong, Y.; Sheen, J. (2014) TOR signaling networks in plant growth and metabolism. Plant Physiol.

120.	Xu Q, Chen S, Ren Y. (2018) Regulation of Sucrose Transporters and Phloem Loading in Response to Environmental Cues. Plant physiology. 176:930–945. pmid:29158330

121.	Xu Q, Yin S, Ma Y, Song M, Liesche J. (2020) Carbon export from leaves is controlled via ubiquitination and phosphorylation of sucrose transporter SUC2. Proceedings of the National Academy of Sciences. 117(11): 201912754. pmid:32123097

122.	Xu, Q. et al. (2020) Carbon export from leaves is controlled via ubiquitination and phosphorylation of sucrose transporter SUC2. Proc. Natl. Acad. Sci. U. S. A. 117, 6223–6230

123.	Yadav, U.P.; Ivakov, A.; Feil, R.; Duan, G.Y.; Walther, D.; Giavalisco, P.; Piques, M.; Carillo, P.; Hubberten, H.M.; Stitt, M.; et al (2014). The sucrose–trehalose 6-phosphate (Tre6P) nexus: Specificity and mechanisms of sucrose signalling by Tre6P. J. Exp. Bot.65, 1051–1068.

124.   Yuan, X., Xu, P., Yu, Y., & Xiong, Y. (2020). Glucose-TOR signaling regulates PIN2 stability to orchestrate auxin gradient and cell expansion inArabidopsisroot. Proceedings of the National Academy of Sciences, 117(51), 32223–32225. https://doi.org/10.1073/pnas.2015400117

125.   Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RAC, Powers SJ, Schluepmann H, Delatte T, Wingler A, Paul MJ. (2009). Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. Plant Physiology 149, 1860–1871

126.   Zhang, S., Peng, F., Xiao, Y., Wang, W., & Wu, X. (2020). Peach PpSnRK1 Participates in Sucrose-Mediated Root Growth Through Auxin Signaling. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.00409.