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TOR-SnRK1 are modulated by upstream signaling to regulate growth and development in vitro of 'Myrobalan 29C' plum rootstock

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Abstract – The aim of this study was: (*i*) understand how upstream signaling modulated TOR-SnRK1 nexus; and (*ii*) establish an interplay between SnRK1-TOR nexus, sugar availability, sucrolytic enzyme activities, expression level of key genes related to signalling and sugar metabolism, including trehalose, in *in vitro*-grown of 'Myrobalan 29C'plum rootstock (*Prunus cerasifera*). Explants were cultivated in Murashigue and Skoog medium (MS) with trehalose (0; 1,0 and 10 mM). In 3 days, the antagonistic role of *PcSnRK1* and *PcTOR* was confirmed in plants treated with 10mM trehalose, possibly indicating that 'Myrobalan 29C' was not in a stress condition. Furthermore, a *PcTREA* up-regulation was observed, which can lead to glucose accumulation, that in turn is precursor of sorbitol synthesis. Regarding the growth parameters evaluated after 21 days of *in vitro* culture, the uppermust number of shoots and explant length was observed at 10mM trehalose. Such positive response may be due to an increase in Glucose and UDP-Glc content, direct products of sucrose synthase (SuSy) enzyme. Consistent with these results, the highest availability of these molecules may be the upstream signal for TOR-activation. Interestingly, in this same condition, a sucrose accumulation was observed, which may also have contributed to *PcTOR* up-regulation and ameliorate in growth parameters.

Index Terms: *Prunus cerasifera*, rootstock, *in vitro* culture, trehalose, sugar sensing/signaling.

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TOR-SnRK1 são modulados por uma sinalização upstream para regular o crescimento e o desenvolvimento in vitro do porta-enxerto de ameixeira 'Myrobalan 29C'

Resumo – O objetivo deste estudo foi: (*i*) compreender como a sinalização *upstream* modula o nexo TOR-SnRK1; e (*ii*) estabelecer uma interação entre o nexo SnRK1-TOR, disponibilidade de açúcar, atividades de enzimas sucrolíticas, nível de expressão de genes-chave relacionados à sinalização e metabolismo de açúcar, incluindo trealose, em porta-enxertos de ameixeira 'Myrobalan 29C' (*Prunus cerasifera*) cultivados *in vitro*. Explantes foram cultivados em meio Murashigue e Skoog (MS) com trealose (0; 1,0 e 10 mM). Aos 3 dias, o papel antagônico de *PcSnRK1* e *PcTOR* foi confirmado em plantas tratadas com trealose 10mM, possivelmente indicando que 'Myrobalan 29C' não estava em uma condição de estresse. Além disso, foi observada uma regulação positiva do gene *PcTREA*, que pode levar ao acúmulo de glicose, que por sua vez é um precursor da síntese de sorbitol. Em relação aos parâmetros de crescimento avaliados após 21 dias de cultivo *in vitro*, o maior número de brotações e de comprimento do explante foi observado em resposta a trealose 10mM. Tais respostas positivas podem ser devido ao aumento do teor de glicose e UDP-Glc, produtos diretos da enzima sacarose sintase (SuSy). Consistente com este resultado, a maior disponibilidade dessas moléculas pode ser o sinal *upstream* para ativação de TOR. Interessantemente, nesta mesma condição, foi observado um acúmulo de sacarose, o que também pode ter contribuído para o aumento da expressão do gene *PcTOR* e para a melhora nos parâmetros de crescimento.

Termos para indexação: *Prunus cerasifera*, porta-enxertos, cultivo *in vitro*, trealose, percepção/sinalização de açúcar.

Introduction

Sugars regulate many aspects of plant growth and development. In this sense, the trehalose (non-reducing disaccharide) is used as an energy source, as storage and transport molecule for glucose, and despite of the physiological effects it is found in very low amounts in plants, pointing to a role in the signaling process (TSAI et al. 2016). Trehalose is synthesized from UDP-glucose and glucose-6-phosphate by trehalose-phosphate synthase (TPS) and then dephosphorylated by trehalose-phosphate phosphatase (TPP) and can be hydrolysed to glucose by trehalase (TREA) (CABIB; LELOIR,1958). Some studies have shown the role of the precursor of trehalose, trehalose-6-phosphate (T6P), as an important signaling molecule (FIGUEROA et al. 2018) that regulates growth and development, however, the regulation mechanism is unknown. Recent studies have shown that T6P inhibits the activity of the Sucrose non-fermenting-1 (SNF1)-relatedkinase 1 (BAENA-GONZALEZ et al. 2017, 2020; WINGLER; HENRIQUES, 2022).

According to Fichtner et al. (2021) SnRK1 and Target of Rapamycin (TOR) are conserved protein kinases that act as sensors of energy availability in order to maintain energy homeostasis. The TOR is activated in favorable conditions, while SnRK1 under nutrient and energy starvation (TOMÉ et al. 2014; MARGALHA et al. 2019). The TOR-SnRK1 nexus has emerged as crucial in regulating

the perception and responses to energy/sugar levels in the cells/tissues (RODRIGUEZ et al. 2019) and to adjust plants to the environments (BAENA-GONZALEZ; HANSON, 2017).

Although it is widely accepted that both kinases (SnRK1 and TOR) exert control on growth and stress responses (MARGALHA et al. 2019; WINGLER; HENRIQUES, 2022), an overview of regulatory networks on its activation mechanisms is still missing. In a sense, the in vitro plant culture, through the possibility of manipulating the culture medium, may help to define the physiological, biochemical and molecular roles of trehalose and the complexity of this regulation exerts on putative targets, such as SnRK1 and TOR. In contrast with the advances made in discovering functions of TOR-SnRK1 nexus, the upstream signals of TOR-SnRK1 remain widely unknown (SONG et al. 2021), as well as the role of trehalose as a mediating molecule of perception and signaling in plants. To fill this gap, the aim of this study was: (i) understand how upstream signaling modulated TOR-SnRK1 nexus; and (ii) establish an interplay between SnRK1-TOR, trehalose metabolism, enzyme activity and their associated sugar content in in vitro-grown 'Myrobalan 29C'plum rootstock (*Prunus cerasifera*).

Material and methods

As a plant material 'Myrobalan 29C (*Prunus cerasifera*) rootstock were used. This rootstock is mainly used for Japanese Plum and Prune, and it was selected in California from an open-pollinated seedling of *P. cerasifera*. It has as main characteristics the resistance to root-knot nematode (*Meloidogyne spp*.), grow vigorously and makes large tree with low suckers and good anchorage, promoting good yields and fruit quality (OKYE et al. 1987). Explants (2.0 cm long) were cultivated in flasks containing MS (MURASHIGUE; SKOOG, 1962) medium and different trehalose concentrations (0, 1 and 10 mM) supplemented with BAP 0.4 mg L^{-1}, IBA 0.05 mg L^{-1} and GA_3 0.3 mg L^{-1} of, 7 g L^{-1} agar and pH 5.2. In vitro cultures were incubated in a growth room (25 ± 2°C) with a photosynthetic photon flux density of 48 µmol photon m^{-2} s^{-1} and 16 h light/8 h dark.

After 3 and 21 days, leaves were collected for biochemical and molecular analysis. The number and lenght of shoots and leaves were assessed in a non-destructive way. For gene expression analysis, total RNA was obtained by the Lithium Chloride method modified by Chang et al. (1993). Total RNA was isolated, quantified and checked quality and integrity. Two micrograms of total RNA were used to obtain cDNA with a final volume of 20 µL. Primers were designed based on coding sequences of *Prunus persica* deposited in the Genome Database for Rosaceae (https://www.rosaceae.org/), using the Primer Designing tool, from the NCBI database, and in detail described in **Table 1**. The reference gene *Elongation factor 1-α* (*EF 1-α*) was applied for data normalization, according to previous studies. The gene expression results were presented as a heatmap (**Figure 1**). The relative quantification (RQ) was calculated with the $2^{-\Delta\Delta Ct}$ method (LIVAK; SCHMITTGEN, 2001). Three technical repetitions were performed for each biological replicate, including samples for the control treatment as template-free controls.

For quantification of starch, the method used was described by Graham and Smydzuk (1965), while that for sucrose was proposed by Handel (1968), with some modifications. Absorbance readings for Starch and Sucrose were determined at 620nm using a Ultrospec® 7000/7000PC UV–Visible spectrophotometer. Besides, for the quantification of starch, the values obtained for soluble sugar total were corrected by factor 0.9, according to McCready et al. (1950). Additionally, sucrolytic enzymes activities were measured. For that, leaves samples from the explants (about 0.4 g) were ground until a fine powder. The extraction of the neutral/alkaline invertase (CINV) and the acid invertase enzymes (CWINV and VINV) followed the methodology described by Zeng et al. (1999), with minor modifications. The supernatant solution was collected to measure soluble invertase activity (VINV

Table 1 - Primer sequences to RT-qPCR analysis of the genes assayed in plum rootstocks 'Myrobalan 29C'.

GENE	Putative name	Primer foward (5'-3')	Primer reverse (5'-3')	Reference	Tm
CINV1	Alkaline/neutral invertase1	TGAATGGTGAGCCTGAGA	GGATAGGGTCGTGAAGAA	(1)	82.0
CINV2	Alkaline/neutral invertase2	TATGATTGATAGACGGATGG	CTAAGTCGGTTATTGATTGC	(1)	80.5
Susy	Sucrose synthase	ATTGGAAATGGCGTTGAGT	TTGCCCTTGTAGCAGTGAA	(1)	82.5
CWINV	Cell wall acid invertase	GTCACAGCAGCACAGGCAG	CCAATACGAGTAACCCGAAT	(1)	81.0
HXK	Hexokinase 1	AGATGTGGTGGGAGAGCTGA	GTGCCCAATATCACAGCAGC	-	80.5
SPS	Sucrose phosphate synthase	CATACCCCAAACACCACAA	TATCAACAGGACCCCC	(1)	80.0
S6PDH	Sorbitol-6-phosphate dehydrogenase	ACATGGCACGACATGGAAAAGAC	AATTGGCTCACTTGAGGCTTGAT	(2)	80.5
SDH	Sorbitol dehydrogenase	CGAAGTTGGTAGCTTGGTGAAGA	CTTGCACTGCTCACATCTCCA	(2)	83.5
TPP	trehalose phosphate phosphatase	GTCGAGCACCCTTCTGCATT	TTGGTGAAAGGGTCCCATCG	-	79.5
TPS	trehalose phosphate synthase	AGAACACCGCGGTCTCATTT	ATTGGGCCTGTCCACAGATG	-	79.0
TREA	trehalase	CAACAAAAGCCTCGTCAGCC	TTTTCCAAAAGTGGCGAGCG	-	83.0
SnRK1	Sucrose non-fermenting 1-related kinase1	GCTCTAGAATGGATGGATCGGTTG	GCGTCGACTTAAAGGACCCG	-	79.0
TOR	Target of Rapamycin	TGGAAGAAGAAGCCCGTGAC	TTCTCCGCAACATCACTGCT	-	80.5
EF-1α	Elongantion factor 1-alpha	AATTGCCTTTGTTCCCATCTCTG	TGGGCTCCTTCTAATCTCCTTA	(3)	84

Reference: 1. ZHANG et al. (2013); 2. JIMENEZ et al. (2013); 3. XU et al. (2008).

and CINV) and the precipitate was collected to measure insoluble invertase (CWINV). The aliquots were collected after 10 and 40 min to determine enzymatic activity. Enzymatic activity was evaluated by quantifying reducing sugars produced according to the dinitrosalicylic acid (DNS) method, previously described by Miller (1959). Sucrose Synthase (Susy) and Sucrose Phosphate Synthase (SPS) activity were determined according to Lowell et al. (1989), with some modifications. The protein content was determined using Bradford's (1976) method. Half of the extracts were boiled for 10 min, and the other half were incubated for 1 h at 37°C and then boiled for 10 min. The sucrose formed was measured with the anthrone method (GRAHAM AND SMYDZUK 1965). All enzyme activities were determined in triplicate and expressed in micromoles of glucose per gram of fresh weight per min (µmol glucose g^{-1} FW min^{-1}), except to SPS that was expressed in micromoles of sucrose per gram of fresh weight per hour (µmol sucrose g^{-1} FW h^{-1}).

The experiment was repeated three times and was set up in a completely randomized factorial design with three treatments (trehalose concentrations) and each treatment was constituted by five flasks with four explant per flask. Results correspond to mean ± standard deviation. For statistical analysis, ANOVA and Tukey Test at the 5% probability level ($P < 0.05$) were performed to calculate for significant differences among treatments using the Sisvar 5.0 software (FERREIRA, 2011).

Results and discussion

In spite of the multiple roles that have been proposed to trehalose, such as compatible solutes, stress protecting and regulation of plant growth and development (ALMEIDA et al. 2007; LLORENTE et al. 2007), limited information are available about the effects that exogenous trehalose induce on morphology, sugar metabolism and signalling in woody fruit trees (in vitro and ex vitro conditions). Furthermore, it is extremely necessary to optimize the amount of trehalose

required to development of in vitro plants. Previous studies showed that trehalose allows the plants to tolerate adverse in vitro conditions (LLORENTE et al. 2007). Thus, the aim of this study was to explore the exogenous Trehalose: as a source energy, carbon storage and sugar signal in 'Myrobalan 29C' after 3 and days 21 of in vitro culture.

The regulation of trehalose biosynthetic genes in response to trehalose-treated in 'Myrobalan 29C' was studied. The highest exogenous trehalose (10mM) provided up-regulation of the *PcTPP* (2.14±0.05) and *PcTREA* genes (2.14± 0.04) after 3 days in vitro culture, on the other hand, *PcTPS* (0.55±0.08) and *PcSnRK1* (0.18±0.07) were down-regulated (**Figure 1**). Trehalose-6-phosphate (T6P) and SnRK1 inhibits Susy and SPS activity (FEDOSEJEVS et al. 2018; WANG et al. 2022).

In our experiment, *PcTPS* and *PcSnRK1* were down-regulated, possibly contributing to regulation of *PcSusy* and *PcSPS* genes. Extensive studies have demonstrated that sugar signals can be translated by protein kinases, such SnRK1 and TOR (SONG et al. 2021, WINGLER; HENRIQUES, 2022). In this study, the expression patterns of *PcSnRK1* and *PcTOR* genes were down- and up-regulated in trehalose 10mM, supporting their globally antagonistic roles (MARGALHA et al. 2019; FICHTNER et al. 2021). SnRK1 is activated in response to energy decline; conversely, TOR is activated and promotes growth and biosynthetic processes in high-energy availability (Figure 3) (BAENA-GONZALEZ; HANSON, 2017; WINGLER; HENRIQUES, 2022).

Taking into account the earlier studies and the results of our experiment, it is possible to infer that in this condition 'Myrobalan 29C' was not in a stress condition (by sugar supply), especially because it was in the culture medium for 3 days and with the supplementation of 10mM of trehalose. Although this study focused more on the effect of the trehalose on the signaling process, it is impossible to rule out its role as a stress-protective molecule since it is also considered a compatible solute (KOSAR et al. 2019).

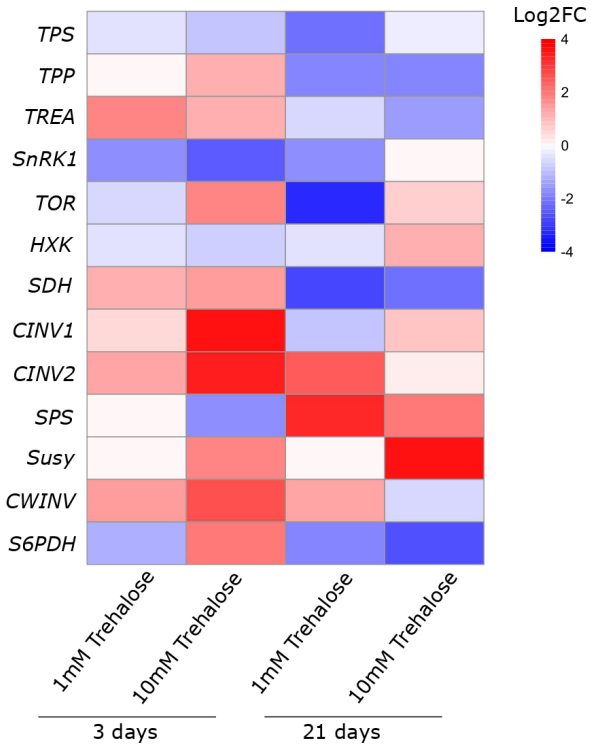

Figure 1 – Heatmap showing the expression levels of genes involved in sugar signaling and carbohydrate metabolism in 'Myrobalan 29-C' plum rootstocks cultured in vitro with different concentrations of trehalose (0, 1, and 10mM) for 3 and 21 days. The color scale refers to Log2FC. The red scale refers to up-regulated genes and the blue scale refers to down-regulated genes.

The increased expression of *PcTREA* gene (trehalose degradation) observed in this study can lead to glucose accumulation and signaling to activate *PcTOR,* that in its turn had the expression increased 3-fold in this condition. Besides, this accumulation of glucose, can be used as precursor for sorbitol synthesis. In the Rosaceae family, sorbitol represents the main form of carbon transported from source to sink tissues (YANG et al. 2018). Sorbitol-synthesizing species raises an interesting question as to how the trehalose interact with sugar signalling and metabolism in woody fruit trees, since the available information is mostly in Arabidopsis and cereal crops. Genes encoding the key enzymes of sorbitol synthesis (S6PDH) and degradation (SDH) were measured. Our results showed an up-regulation of *PcS6PDH* (4.07±0.40) and *PcSDH* (2.78±0.06*)* genes in response to trehalose 10mM. This revealed

that exogenous trehalose influences on sorbitol metabolism, however, further studies are needed to better unravel its effects.

Zhang et al. (2017) suggested that Tre6P is more closely related to sorbitol than other soluble sugars. A more recent study showed that SnRK1 is involved in sorbitol metabolism in peach fruits, in which the SnRK1 activated SDH (sorbitol dehydrogenase), and it also regulated the activities of SuSy (sucrose synthase) and SPS (sucrose phosphate synthase), enhancing sucrose accumulation (YU et al. 2021). Regarding to glycolytic enzymes, *PcHXK* gene expression was stable. Based on that, glucose and fructose can be accumulated in cytoplasm or can also be stored in the vacuoles, rather than converted to hexose-phosphate (G6P and F6P) for ATP production via glycolysis. This suggestion is further supported by up-regulation of the *PcTREA*, sucrose hydrolytic enzymes (*PcCINV1, PcCINV2* and *PcCWINV*), and *PcSusy* (cleavage) that were up-regulated in response to trehalose 10mM. On the oth-

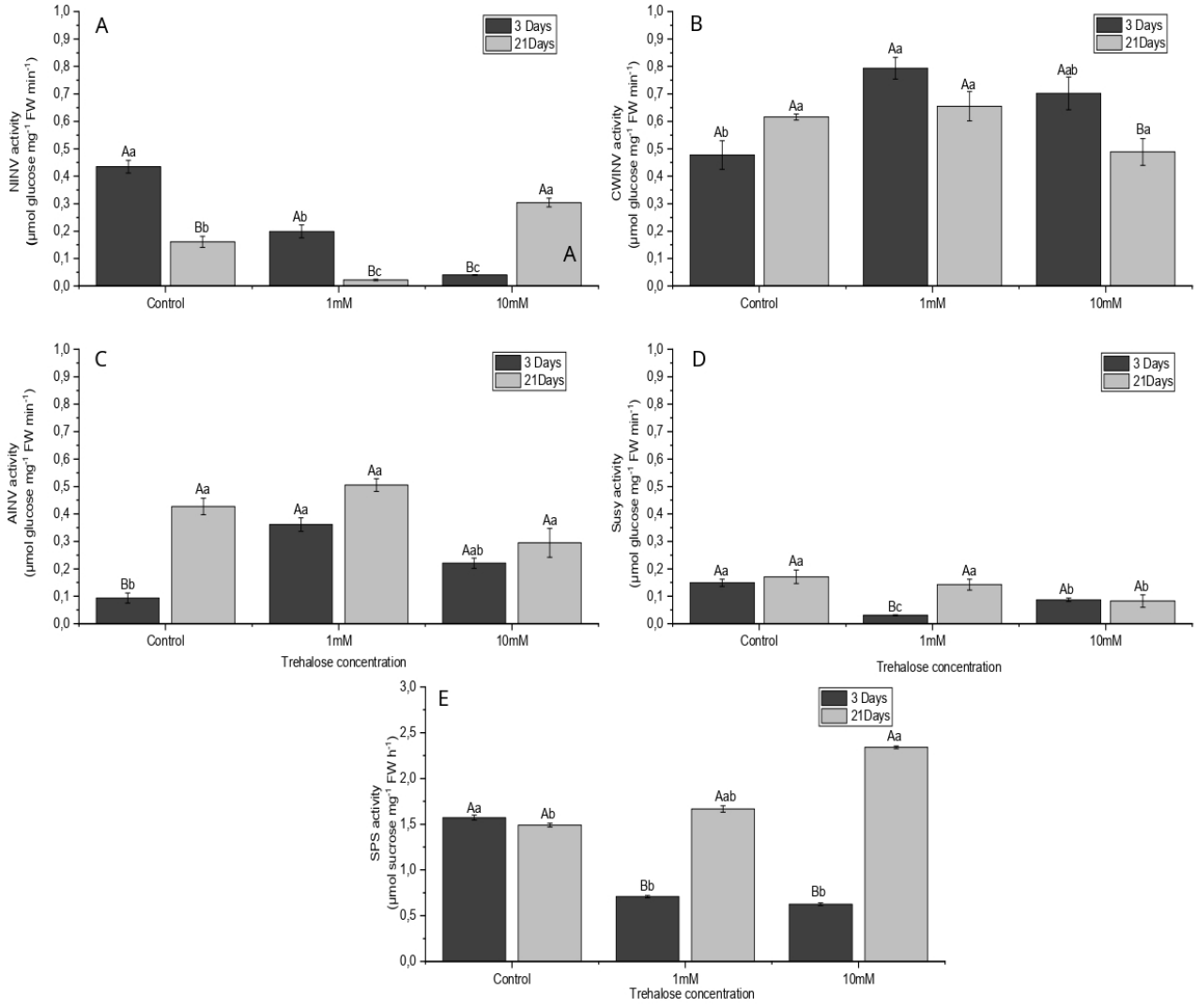

Figure 2 – Activity determination of sucrose synthesis and degradation enzymes in 'Myrobalan 29-C' plum rootstocks cultured in vitro with different concentrations of trehalose (0, 1 and 10mM) for 3 and 21 days. (A) neutral/alkaline invertase (NINV), (B) cell wall acid invertase (CWINV), (C) Vacuolar acid invertase (AINV), (D) sucrose synthase (Susy) and (E) sucrose phosphate synthase (SPS). Error bars represent the standard deviation (*n*=3). Columns with different uppercase letters indicate differences between times (3 and 21 days) for the same concentration, while different lowercase letters indicate differences between concentrations for the same time. Significant differences based on ANOVA followed by Tukey test P≤0.05.

er hand, it is not supported by their activity (**Figure 2A-C**).

T6P (intermediate of trehalose biosynthesis) plays a key control between low carbon and high carbon signaling in the form of sucrose (GRIFFITHS et al. 2016; ZHANG et al. 2017). Overall, the T6P accumulation occurs in response to high sucrose content (SCHLUEPMANN et al. 2011). In our study no differences were observed between treatments for sucrose content after 3 days of the in vitro culture, suggesting that the levels observed were not sufficient to provide an up-regulation of *PcTPS* gene (synthesis enzyme of T6P). Starch metabolism is one of the most striking examples of regulation by trehalose (PAUL et al. 2008). In this sense, trehalose has been shown to regulate starch breakdown in plastids (PONNU et al. 2011) and T6P seems to activate the ADP-Glc pyrophosphorylase (AGPase) enzyme (thioredoxin-dependent redox mechanism), in response to high sucrose (SCHLUEPMANN et al. 2011). Interestingly, our results show a decrease and stability in starch and sucrose content, respectively (**Table 2**) which may be related to low T6P levels and with *PcTPP* up-regulation (**see chloroplast in Figure 3**).

Regarding the growth parameters, the greatest number of shoots and explant length was observed at 10mM trehalose (**Table 3**). Similar results were found to Llorent et al. (2007) in *Simmondsia chinensis* where trehalose promoted the shoot growth. This improved in plant growth in response to trehalose may be due to increase in Glucose and

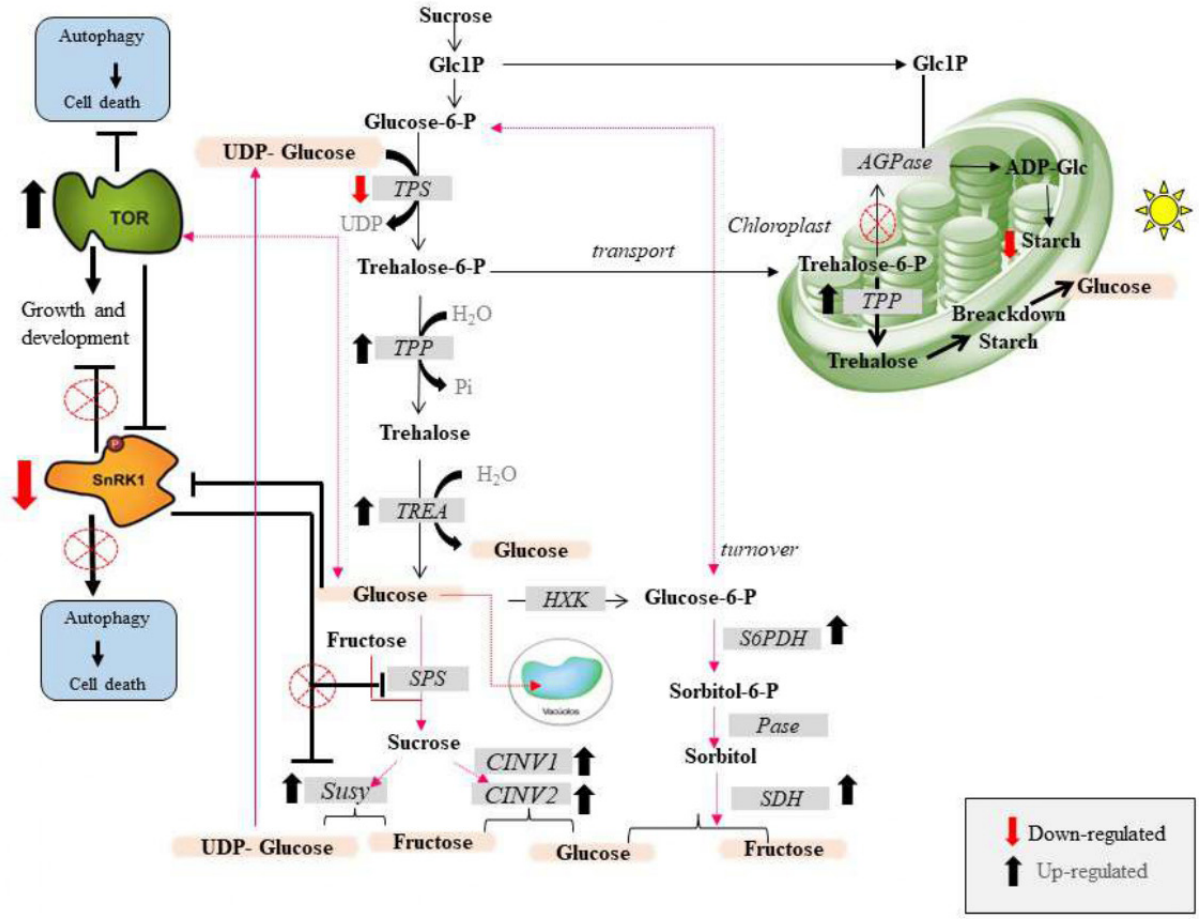

Figure 3 - Regulatory loop showing interplay between trehalose metabolism in different organelles and SnRK1-TOR nexus in 'Myrobalan 29-C' plum rootstocks cultured in vitro with trehalose 10mM for 3 days. Enzymatic flow is depicted as arrows from substrates to products. Enzymes catalyzing each step are shown into each arrow. Upward arrows in black indicated up-regulation, whereas those red downward arrows represent down-regulation.

UDP-Glc content, direct products of SuSy enzyme. In our study showed *PcSusy* gene was up-regulated about 12-fold (12.69±0.38) when compared to the control plants to same condition (**Figure 1**). However, an increase in Susy activity was not observed (**Figure 2D**).

The sucrose content was about 6-fold higher than that observed in the control plants (**Table 2**), in response to 10mM trehalose, what may have contributed to the up-regulation of *PcTOR* gene (**Figure 1**). TOR which in turn positively regulates the growth and development in response to high sugar availability (RODRIGUEZ et al. 2019). The causal relationship between high sucrose content, *PcTOR* gene upregulated, and ameliorates in growth parameters supported this statement (**Figure 3**). Besides, Glc and sucrose are activators of TOR signaling (LI et al. 2017) and responsible for development of shoot apical (SONG et al. 2021). In a physiological context, Glc-TOR signaling activates Brasinosteroids (plant hormone) pathway by phosphorylates BIN2, a negative regulator of Brasinosteroids (BR), thus promoting growth (ZHANG et al. 2016). Overall, the highest availability of sucrose and glucose may be the upstream signal for TOR-activation (hexose-signal) and an indirect repressor of SnRK1 (**Figure 4**). Thus, our data also are consistent with the hypothesis that SnRK1 is not activated and therefore it is not blocking the in vitro growth and development of shoots of 'Myrobalan 29C' (**Figure 3**).

The increase in sucrose content observed in this study is accompanied to increase in *PcSPS* gene expression and activity, and additionally of the up-regulation of the *PcSusy* gene (about 12-fold). The products of sucrose cleavage by SuSy are available for many metabolic pathways, in case, UDP-Glc and ADP-Glc to trehalose and starch synthesis, respectively. Although UDP-Glc be the main nucleotide phosphate, ADP-Glc also is product of Susy (BAROJA-FERNANDEZ et al. 2012). Some evidence suggesting that Susy is involved in starch synthesis pathway, and Stein and Granot et al. (2019) proposed a model in which starch accumulation is determined by SuSy activity. Based on this, we suggested that the increasing in starch

Table 2 - Sugar content in in 'Myrobalan 29-C' plum rootstocks cultured in vitro with different concentrations of trehalose (0. 1 and 10mM) for 3 and 21 days.

Trehalose (mM)	Soluble Sugar total		*Sucrose		Starch	
	3 days	21 days	3 days	21 days	3 days	21 days
0	50.44^{ns}	47.98^{ns}	0.20±0.04Aa	0.21±0.00Ab	9.48±0.15Aa	0.49±0.14Bb
1			0.16±0.01Aa	0.12±0.01Ab	2.78±0.08Bb	6.05±0.09Aa
10			0.22±0.03Ba	1.20±0.14Aa	2.70±0.29Bb	5.55±0.14Aa
CV%	23.31		22.91		24.90	

*mg/g^{-1} MF

Columns with different capital letters indicate differences between times (3 and 21 dys) for the same concentration, while different lowercase letters indicate differences between concentrations for the same time. Significant differences were based on ANOVA, followed by the Tukey test at P ≤ 0.05.

Table 3 - Growth parameters evaluated in 'Myrobalan 29-C' 'Myrobalan 29-C' plum rootstocks cultured in vitro with different trehalose concentrations (0, 1 and 10mM) for 21 days.

Trehalose (mM)	*Shoot number	*Leaf number	*Leaf lenght (cm)	* Explant lenght (cm)
0	2.778±0.064 b	27.333^{ns}	1.446^{ns}	2.294±0.047 b
1	3.333±0.112 ab			2.289±0.053 b
10	4.334±0.192 a			2.644±0.017 a
VC%	11.51	2.99	8.48	5.22

Different lowercase letters in the column represent differences between concentrations. Significant differences based on ANOVA followed by Tukey test P≤0.05. *per explant

content observed in this condition is related with sucrose cleavage by Susy that yields ADP-Glc. Overall, our results implied that Susy was acting as a cleavage enzyme and sucrose synthesis occurring by SPS pathway. In recent studies, Wang et al. (2022) pointed that accumulated sucrose in peach trehalose-treated is associated with the decrease in expression of *PpSnRK1* and increases in the expression and activity of the SPS (**Figure 1 and 2E**). This is in agreement with the findings of this study.

Regarding the expression of trehalose biosynthetic genes, it was possible to observe in response to 10mM trehalose, the *PcTPP* (0.28 ± 0.03) and *PcTREA* (0.35 ± 0.04) genes were down-regulated, on the other hand, *PcTPS* and *PcSnRK1* genes showed stability in their expression (**Figure 1**). According to Schluepmann et al. (2011) and Griffiths et al. (2016) T6P functions as an inhibitor of the kinase SnRK1. However, the molecular mechanisms thereof remain unknown (PEIXOTO; BAENA-GONZÁLEZ, 2022; ONWE et al. 2022). Our results therefore suggest that the lack of T6P caused by the stable expression of the synthesis gene (*PcTPS*) observed in this study did not provide the inhibition of *PcSnRK1* in 'Myrobalan 29C'. Besides, SnRK1 up-regulated is related with low-energy responses (TOMÉ et al. 2014; BAENA-GONZALEZ; LUNN, 2020). Interestingly, no harmful effects were observed in the explants growth in this condition (**Table 3**), supporting the hypothesis that the explants are in optimal growth conditions and promoting processes for carbon utilization and anabolism. Overall, T6P/SnRK1 are essential on regulation plant growth and development as proposed by GRIFFITHS et al. (2016).

Taking all the results obtained in the present study, we suggest that trehalose not only interacts with the sucrose, but also with sorbitol and starch metabolism. Furthermore, we postulate for the first time the mechanism that SnRK1-TOR nexus uses to modulate together with trehalose the growth of sorbitol-synthesizing species. Future studies will be needed to evaluate the role of trehalose and of the regulators of energy homeostasis, SnRK1-TOR in plants under stress conditions (heat temperature, drought, flooding and

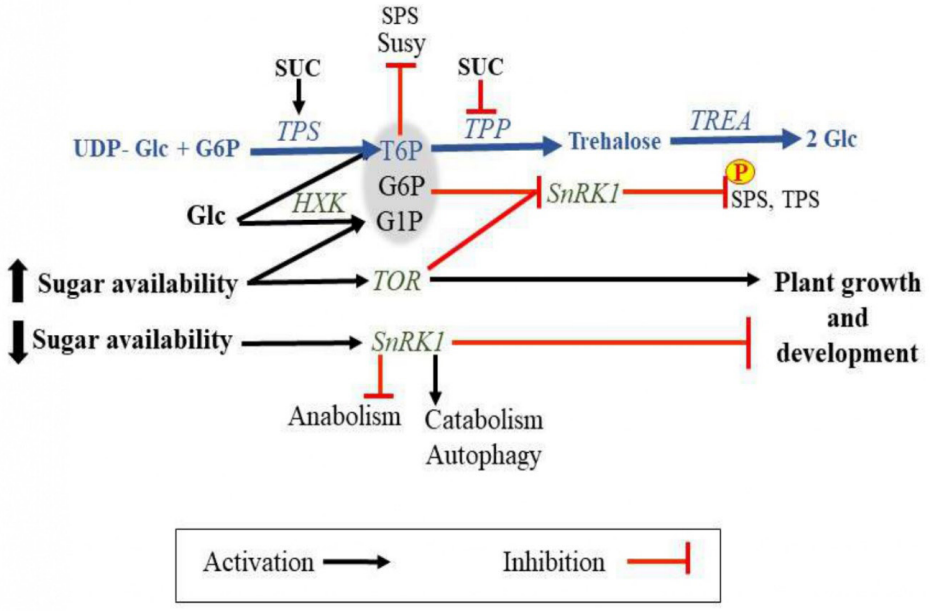

Figure 4 - Overview of regulatory networks to low and high carbon (signaling pathways) and trehalose metabolism in 'Myrobalan 29C'. SnRK1 activity is found to be inhibited by diverse sugar phosphates, including trehalose-6-phosphate (T6P). In addition, T6P inhibits Susy and SPS activity. TOR-SnRK1 nexus signals to permissive or restrictive growth decisions. Trehalose synthesis involves a twostep, catalysed by trehalose-6-phosphate synthase (TPS) and trehalose 6-phosphate phosphatase (TPP) and degraded by trehalase (TREA) (In blue).

salinity), since it is accepted in the literature that SnRK1-TOR mechanism operates differently in these conditions (MARGALHA et al. 2019; RODRIGUEZ et al. 2019).

Conclusion

The glucose is an upstream signal to SnRK1-TOR in order to maintain in vitro growth and development of 'Myrobalan 29C' plum rootstock in response to exogenous application of trehalose. Besides, the sorbitol metabolism was modulated by exogenous trehalose.

The regulatory loop, which involves trehalose and starch metabolism in different organelles and the SnRK1-TOR nexus has been proposed. Exogenously applied trehalose do not cause harmful effects in growth and developmental of 'Myrobalan 29C' plum rootstock. Remarkably this may be due to finely tuned of SnRK1-TOR.

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References

ALMEIDA, A.M.; CARDOSO, L.A.; SANTOS, D.M.; TORNÉ, J.M.; FEVEREIRO, P.S. Trehalose and its applications in plant biotechnology. **In Vitro Cellular & Developmental Biology – Plant**, Wallingford, v.43, p.167-77, 2007.

BAENA-GONZALEZ, E.; HANSON, J. Shaping plant development through the SnRK1–TOR metabolic regulators. **Current Opinion in Plant Biology**, Oxford, v.35, p.152-7, 2017.

BAENA-GONZALEZ, E.; LUNN, J.E. SnRK1 and trehalose 6-phosphate – two ancient pathways converge to regulate plant metabolism and growth. **Current Opinion in Plant Biology**, Oxford, v.5, p.52-9, 2020.

BAROJA-FERNANDEZ, E.; MUNOZ, F.J.; LI, J.; BAHAJI, A.; ALMAGRO, G.; MONTERO, M.; POZUETA-ROMERO, J. Sucrose synthase activity in the sus1/sus2/sus3/sus4 Arabidopsis mutant is sufficient to support normal cellulose and starch production. **Proceedings of the National Academy of Sciences**, Washington, v.109, n.1, p.321-6, 2012.

LLORENTE, E.; JUAREZ, L.M.; APÓSTOLO N.M. Exogenous trehalose affects morphogenesis in vitro of jojoba. **Plant Cell, Tissue and Organ Culture**, Dordrecht, v.89, p.193-201, 2007

BRADFORD, M.M. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dy binding. **Analytical Biochemistry**, New York, v.72, p.248-54, 1976.

CABIB, E.; LELOIR, L.F. The biosynthesis of trehalose phosphate. **International Journal of Biological Chemistry**, New York, v.231, p.259-75, 1958.

CHANG, S.; PURYEAR, J.; CAIRNEY, J. A simple and efficient method for isolating RNA from pine trees. **Plant Molecular Biology Reporter**, Athens, v.11, p.113-6, 1993.

FERREIRA, D.F. SISVAR: um sistema computacional de análise estatística. **Ciência e Agrotecnologia**, Lavras, v.35, p.1039-42, 2011.

FEDOSEJEVS, E.T.; FEIL, R.; LUNN, J.E.; PLAXTON, W.C. The signal metabolite trehalose-6-phosphate inhibits the sucrolytic activity of sucrose synthase from developing castor beans. **FEBS Letters**, Amsterdam, v.592, p.2525-32, 2018.

FICHTNER, F.; DISSANAYAKE, I.M.; LACOMBE, B.; BARBIER, F. Sugar and nitrate sensing: a multi-billion-year story. **Trends in Plant Science**, Kidlington, v.26, n.4, p.352-74, 2021.

FIGUEROA, C.M.; LUNN, J.E. A tale of two sugars: trehalose 6-phosphate and sucrose. **Plant Physiology**, Oxford, v.172, p.7–27, 2018.

GRAHAM, D.; SMYDZUC, J. Use of anthrone in the quantitative determination of hexose phosphates. **Analytical Biochemistry**, London, v.11, p.246-55, 1965.

GRIFFITHS, C.A.; PAUL M.J.; FOYER C.H. Metabolite transport and associated sugar signalling systems underpinning source/sink interactions. **Biochimica et Biophysica Acta (BBA) - Bioenergetics**, Amsterdam, v.1857, n.10, p.1715-25, 2016.

HANDEL, E.V. Direct microdetermination of sucrose. **Analytical Biochemistry**, London, v.22, p.280-3, 1968.

JIMÉNEZ, S.; DRIDI, J.; GUTIÉRREZ, D.; MORET, D.; IRIGOYEN, JJ.; MORENO, MA.; GOGORCENA, Y. Physiological, biochemical and molecular responses in four Prunus rootstocks submitted to drought stress. **Tree Physiology**, Oxford, v.33, p.1061-75, 2013.

KOSAR, F.; AKRAM, N.A.; SADIQ, M.; AL-QURAINY, F.; ASHRAF, M. Trehalose: a key organic osmolyte effectively involved in plant abiotic stress tolerance. **Journal Plant Growth Regulation**, New York, v.38, n.2, p.606-18, 2019.

LI, X.; CAI, W.; LIU, Y.; LI, H.; FU, L.; LIU, Z.; XU, L.; LIU, H.; XU, T.; XIONG, Y. Differential TOR activation and cell proliferation in Arabidopsis root and shoot apexes. **Proceedings of the National Academy of Sciences**, Washington, v.114, p.2765-70, 2017.

LIVAK, K.J.; SCHMITTGEN, T.D. Analysis of relative gene expres-sion data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. **Methods**, San Diego, v.25, n.4, p.402-8 2001.

LOWELL, C.A.; TOMLINSON, P.T.; KOCH, K.E. Sucrose-metabolizing enzymes in transport tissues and adjacent sink structures in deceloping citrus fruit. **Plant Physiology**, Oxford, v.90, p.1394-402, 1989.

McCREADY, R.M.; GUGGOLS, J., SILVEIRA, V.; OWENS, H.S. Determination the starch and amilose in vegetables.Applications to pea. **Analytical Chemistry**, Washington, v.22, p.1156-8, 1950.

MILLER, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical Chemistry**, Washington, v.31, p.426-8, 1959.

MURASHIGE, T.; SKOOG, F.A. Revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiologia Plantarum**, Oxford, v.15, p.473-97, 1962.

OKIE, W.R. Plum rootstock. *In:* ROM, R.C.; CARLSON, R.F. **Rootstock for fruit crops**. New York: Wiley & Sons, 1987. p.321-60.

ONWE, R.O.; ONWOSI, C.O.; EZUGWORIE, F.N.; EKWEALOR, C.C.; OKONWO, C.C. Microbial trehalose boosts the ecological fitness of biocontrol agents, the viability of probiotics during long-term storage and plants tolerance to environmental-driven abiotic stress. **Science of The Total Environment**, Amsterdam, v.806, p.150432, 2022.

PAUL, M.J.; PRIMAVESI, L.F.; JHURREEA, D.; ZHANG, Y. Trehalose metabolism and signaling. **Annual Review of Plant Biology**, Palo Alto, v.59, p.417-41, 2008.

PEIXOTO, B.; BAENA-GONZÁLEZ, E. Management of plant central metabolism by SnRK1 protein kinases. **Journal of Experimental Botany**, Oxford, v.73, n.20, p.7068-82, 2022.

PONNUJ.; WAHL, V.; SCHMID, M. Trehalose-6-phosphate: connecting plant metabolism and development. **Frontiers in Plant Science**, Lausanne, v.2, p.70, 2011.

RODRIGUEZ, M.; PAROL, R.; ANDREOL, S.; PEREYRA, C.; MARTÍNEZ-NOËL, G. TOR and SnRK1 signaling pathways in plant response to abiotic stresses: Do they always act according to the "yin-yang" model. **Plant Science**, Clare, v.288, p.110220. 2019.

SCHLUEPMANN, H.; BERKE, L.;SANCHEZ-PEREZ, G.F. Metabolism control over growth: a case for

trehalose-6- phosphate in plants. **Journal of Experimental Botany**, Oxford, v.63, n.9, p.3379-90, 2012.

SONG, Y.; ALYAFEI, M.S.; MASMOUDI, K.; JALEEL, A.; REN, M. Contributions of TOR signaling on photosynthesis. **International Journal of Molecular Science**, Basel, v.22, p.8959, 2021.

STEIN, O.; GRANOT, D. An overview of sucrose synthases in plants. **Frontiers in Plant Science**, Lausanne, v.10, p.95, 2019.

TOMÉ, F.; NÄGELE.T.; MATTIA, A.; ABHROOP, GARG.; CARLES, M.; ELLA, N.; LORENZO, P.; ALESSIA, P.; ANDREA, S.; ANNA, T.; MONIKA, T.; MAGDALENA, G. The low energy signaling network. **Frontiers in Plant Science**, Lausanne, v.5, p.353, 2014.

YANG J.; ZHU, L.; CUI.W.; ZHANG, C.; LI D.; MA B, CHENG, L.; RUAN, Y.; MA, F.; MINGJUN, L. Increased activity of MdFRK2, a high affinity fructokinase, leads to upregulation of sorbitol metabolism and downregulation of sucrose metabolism in apple leaves. **Horticulture Research**, Oxford, v.5, p.71, 2018.

WINGLER, A.; HENRIQUES, R. Sugars and the speed of life—Metabolic signals that determine plant growth, development and death. **Physiologia Plantarum**, Copenhagem, v.174, n.2, p.e13656 2022.

YU, W.; PENG, F.; WANG, W.; LIANG, J.; XIAO, Y.; YUAN, X. SnRK1 phosphorylation of SDH positively regulates sorbitol metabolism and promotes sugar accumulation in peach fruit. **Tree Physiology**, Oxford, v.41, p.1077-86, 2021.

XU Y.; ZHANG L.; XIE H.; ZHANG YQ.; OLIVEIRA MM.; RONG-CAI MA. Expression analysis and genetic mapping of three SEPALLATA-like genes from peach (*Prunus persica* (L.) Batsch). **Tree Genetics & Genomes**, Heidelberg, v.4, p.693-703, 2008.

ZHANG C.; SHEN Z.; ZHANG Y.; HAN J.; MA R.; MINGLIANG Y. Cloning and expression of genes related to the sucrose metabolizing enzymes and carbohydrate changes in peach. **Acta Physiologia Plantarum**, Heidelberg, v.35, p.589–602, 2013.

ZHANG, Z.; ZHU, J.Y.; ROH, J.; MARCHIVE, C.; KIM, S.K.; MEYER, C.; SUN, Y.; WANG, W.; WANG, Z.Y. TOR signaling promotes accumulation of bzr1 to balance growth with carbon availability in arabidopsis. **Current Biology**, London, v.25, p.1854-60, 2016.

ZHANG, W.; LUNN, J.E.; FEIL, R.; WANG, Y.; ZHAO, J; Hongxia TAO, H.; GUO, Y.; ZHAO, Z. Trehalose 6-phosphate signal is closely related to sorbitol in apple fruit (Malus domestica Borkh. cv. Gala). **Biology Open**, Basel, v.6, n.2, 2017.

ZENG, Y.; WU, Y.; WAYNE, A.T.; KOCK, K.E. Rapid Repression of maize invertases by low oxygen.invertase/sucrose synthase balance, sugar signaling potential, and seedling survival. **Plant Physiology**, Oxford, v.121, p.599–608, 1999.