Transitioning to the next phase: the role of sugar signaling throughout the plant life cycle

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One sentence summary: Developmental transitions depend on the availability of sufficient carbon resources which is sensed by sugar signaling pathways for high and low carbon availability.
ABSTRACT

Developmental transitions in plants require adequate carbon supply which can be sensed through sugar signaling. Plants have sugar signaling pathways for high carbon availability (including hexokinase-1 (HXK1), trehalose-6-phosphate (T6P), and target of rapamycin (TOR)) and for starvation (including Snf1-related protein kinase-1 (SnRK1) and C/S1 bZIP transcription factors) with interactions between them. If new sinks are created, e.g. during floral transition, high carbon signals are required to progress to the next phase in development while low carbon signals can delay such transitions. Other developmental processes, e.g. seed development, require high and low carbon signals. Recent findings suggest that sugar signaling pathways interact with developmental regulation by microRNA 156 (miR156). For example, T6P interacts with miR156 during the floral transition, but interactions between T6P and miR156 during other developmental transitions have not been described. This Update compares the role of sugar signaling pathways throughout the plant life cycle (seed germination, cotyledon greening, juvenile-to-adult phase transition, floral transition, shoot branching, senescence, and seed development) to identify common and distinct components and their interactions in the context of source-sink relationships.

INTRODUCTION

Developmental transitions in plants result in changes in the source-sink relationship. For example, the growth of new organs can create additional carbon sinks and therefore requires a minimum availability of carbon reserves. If carbon availability is insufficient, there is a risk of premature carbon depletion, resulting e.g. in seedling death, or abortion of fruit or seed development. Sugar signals can regulate developmental transitions and thereby signal if sufficient carbon is available for successful completion of a developmental program. However, the response to sugars and involvement of individual signaling pathways may vary throughout the plant life cycle as sugar signals interact with other developmental and environmental factors.

This Update analyses older and recent findings to identify common and distinct sugar signaling pathways across developmental transitions in the context of changes in the source-sink relationship. Excellent reviews on the role of sugar signaling in plant development have
been published in recent years. Most of these focus on specific signaling pathways or developmental processes; for example, on flowering and interaction with hormones (Matsoukas, 2014a), on growth (Lastdrager et al., 2014), on trehalose-6-phosphate (T6P; O’Hara et al., 2013), on T6P and SnRK1 (Tsai and Gazzarrini, 2014), on hexokinase (Granot et al., 2014), on micro RNAs (Yu et al., 2015), or on SnRK1 and TOR (Baena-González and Hanson, 2017). The whole life cycle of the plant is considered here (Box 1), including step changes (such as floral transition), as well as more gradual processes (such as leaf senescence). Because of space limitations, sugar signaling in root development (Thompson et al., 2017) is not specifically covered. While this Update focuses on sugars as signals for carbon availability, it should be kept in mind that sugar signals also interact with nitrogen (e.g. Osuna et al., 2015; White et al., 2016) and hormone (e.g. Matsoukas, 2014a) signaling pathways.

SUGAR SIGNALING PATHWAYS FOR HIGH AND LOW CARBON AVAILABILITY

In plants, Suc is the predominant sugar formed by photosynthesis, while hexoses (glucose and fructose) are mainly breakdown products of sucrose and starch. The main plant sugar signaling pathways considered in this Update are listed in Box 2.

Hexose accumulation in response to high carbon availability results in the down-regulation of photosynthetic gene expression. This prevents over-investment of nitrogen in the photosynthetic apparatus. Hexose accumulation in plants can be sensed by hexokinase-1 (HXK1) (Granot et al., 2014). Importantly, it was shown that catalytically inactive HXK1 mutant forms still have a signaling function, supporting the view that the role of HKX1 in signaling high sugar availability is independent of its role in glucose metabolism (Moore et al., 2003). More recent work has shown that HXK1 also mediates stomatal closure, thereby contributing to the feedback effect of sugar accumulation on photosynthesis by (Kelly et al., 2012; Kelly et al., 2013).

Only low amounts of the disaccharide trehalose are present in plants (Wingler, 2002). Nevertheless, trehalose-6-phosphate (T6P), the precursor of trehalose in the biosynthetic pathway, has been identified as an important signaling molecule in plants (O’Hara et al., 2013). T6P is synthesized from UDP-Glc and Glc-6-phosphate by T6P synthase (TPS) and hydrolyzed to trehalose by T6P phosphatase (TPP). It accumulates under conditions when Suc content is high and has been proposed to act as a signal for Suc availability (Nunes et al., 2013a; Yadav et al., 2014;
Figueroa and Lunn, 2016). The role of T6P in regulating source-sink interactions during plant development is further discussed in O’Hara et al. (2013) and Griffiths et al. (2016a).

In addition to T6P, the protein kinase target of rapamycin (TOR) signals high carbon availability and stimulates protein translation and plant growth (Deprost et al., 2007; Lastdrager et al., 2014). Despite their common function in promoting growth in response to carbon availability, no direct interaction between T6P and TOR signaling has been identified (Figueroa and Lunn, 2016), which supports the view that T6P is mainly involved in Suc signaling, whereas TOR may be responsible for Glc signaling (Dobrenel et al., 2016), although independently of the HXK1-dependent Glc signaling pathway (Xiong et al., 2013).

In contrast to the growth-stimulating activity of TOR, the Snf1-related protein kinase-1 (SnRK1) inhibits growth at low carbon supply (Lastdrager et al., 2014) and is generally considered to be responsible for signaling low energy availability (Baena-González et al., 2007; Baena-González and Sheen, 2008). The regulation of SnRK1 is complex, including AMP-dependent regulation of its phosphorylation and other post-translational modifications (Crozet et al., 2014), in addition to direct inhibition by T6P and other sugar phosphates (Zhang et al., 2009; Nunes et al., 2013b). T6P inhibits the catalytic activity of SnRK1 in vitro and in vivo (Zhang et al., 2009). However, inhibition of SnRK1 by T6P requires a protein factor that is not present in mature tissues, and the interaction between SnRK1 and T6P is unlikely to be solely responsible for T6P effects (Figueroa and Lunn, 2016). SnRK1 acts at least partially via activation of the C/S1 group of bZIP transcription factors (Baena-González et al., 2007). Recently, a mechanism for this activation has been described: by phosphorylation of the C-group bZIP63, SnRK1 promotes bZIP homo- and hetero-dimerization, e.g. with S-group bZIPs (such as bZIP11) which are not themselves phosphorylated by SnRK1 (Mair et al., 2015). Similar to SnRK1, C/S1 group bZIP transcription factors, whose expression is controlled by sucrose-induced repression of translation (Weltmeier et al., 2009), are involved in reprogramming metabolism in response to low energy supply.

Sugar signaling interacts with microRNAs in regulating development, specifically microRNA156 (miR156) (Matsoukas, 2014b; Yu et al., 2015). High sugar availability results in the down-regulation of miR156 expression and, as a result, increased expression of its targets, the Squamosa Promoter Binding Protein-Like (SBP/SPL) transcription factor genes (Fig. 1). SPL expression, in turn, increases expression of miR172 which leads to the activation of e.g. flowering (Matsoukas, 2014b; Yu et al., 2015; Hyun et al., 2017).
GERMINATION AND COTYLEDON GREENING

Seed reserves are important for seedling establishment, but high external sugar supply (e.g. 6% Glc) inhibits seedling germination and cotyledon greening, resulting in developmental arrest. Mutants in the plastid Glc-6-phosphate/phosphate translocator (GPT2) showed decreased sensitivity to Glc during germination, whereas sensitivity to Glc during cotyledon greening was increased and seedling establishment delayed (Dyson et al., 2014). These results demonstrate that sugar transport across the plastid membrane modulates the sugar response, and also that germination and cotyledon greening are not always regulated by the same pathways.

Developmental arrest of seedling development in response to high sugar supply was used for the isolation of Glc and Suc insensitive mutants (for reviews see Gibson, 2004; Osuna et al., 2015). Abscisic acid (ABA) insensitive (abi) and ABA deficient (aba) mutants have been shown to be insensitive to high Glc concentrations, demonstrating an overlap between ABA and sugar signaling, but sugar signals also interact with ethylene, gibberellin, cytokinin and auxin (Gibson, 2004). In the absence of nitrogen and other inorganic nutrients, a lower concentration of 2% Glc also results in developmental arrest, however in this case without involvement of ABA signaling (Cho et al., 2010). Another Glc-insensitive mutant (gin2-1) harbours a mutation in the gene for the sugar sensor HXK1 (Moore et al., 2003). In the absence of inorganic nutrients, this mutant also shows insensitivity to a low concentration of 2% Glc and can be complemented with catalytically inactive mutant versions of HXK1 (Cho et al., 2010). Similar to the hxl mutation, overexpression of hexokinase-like1 (HKL1) reduces seedling sensitivity to high Glc concentration, suggesting an antagonistic function of HXK1 and HKL1 with respect to seedling development (Karve and Moore 2009; Karve et al. 2012).

T6P interacts with ABA during seedling development. Overexpression of the Arabidopsis T6P synthase gene TPS1 results in reduced Glc and ABA sensitivity, and, in contrast to wild-type plants, TPS1-overexpressing seedlings do not accumulate ABA in response to Glc treatment (Avonce et al., 2004). Mutants with weak TPS1 alleles, on the other hand, are hypersensitive to ABA during germination (Gómez et al., 2010).
TOR expression also decreases sugar and ABA sensitivity (Deprost et al., 2007), indicating that pathways for high sugar availability increase sugar utilization for growth and thereby prevent feedback inhibition of photosynthetic development.

In addition, SnRK1 has been implicated in seed germination and seedling growth in interaction with ABA signaling. In Arabidopsis, overexpression of the SnRK1 gene AKIN10 results in delayed germination (Tsai and Gazzarrini, 2012) and hypersensitivity to Glc and ABA during seedling development (Jossier et al., 2009). SnRK1 acts by phosphorylating and stabilizing FUSCA3 (FUS3) (Tsai and Gazzarrini, 2012); FUS3 promotes ABA synthesis while ABA, in turn, stimulates SnRK1 (Tsai and Gazzarrini, 2014). Overall this leads to ABA-dependent inhibition of seed germination.

While most forms of stress (e.g. drought, cold, salt) lead to sugar accumulation in plants, hypoxia under submergence results in starvation. Anaerobic germination requires mobilization of starch and fueling of fermentation by glycolysis. A rice T6P phosphatase gene (TPP7) was identified as QTL for anaerobic germination tolerance (Kretzschmar et al., 2015). Presence of a functional TPP results in a lower T6P/Suc ratio and increased amylase-dependent starch mobilization for coleoptile growth. These findings, together with the function of SnRK1 for germination under starvation (Lu et al., 2007), are consistent with starvation signals being required for starch mobilization during anaerobic germination and an antagonistic function of T6P and SnRK1. Coleoptile length during germination at low oxygen was decreased in rice hexokinase (HXK7) mutants, but increased in overexpressors (Kim et al., 2016). The authors suggest that HXK7 fuels anaerobic fermentation and that its catalytic function rather than its role in sugar signaling is responsible for anaerobic germination tolerance.

JUVENILE-TO-ADULT PHASE TRANSITION

The juvenile-to-adult phase transition comprises major morphological changes during shoot development (heteroblasty or vegetative phase change), as well as developmental changes that result in the competence to flower under inductive conditions (Poethig, 2010). In some woody species (e.g. Hedera helix, some Acacia and Eucalyptus species), juvenile leaves have a different morphology compared to adult leaves, and some fruit trees (e.g. apple) need to reach a certain age to gain the competence to flower (Poethig, 2013). In the Brassicaceae, the juvenile-to-
adult phase transition enhances the flowering response to inductive long days (Matsoukas et al., 2013) or vernalization (Bergonzi et al., 2013; Zhou et al., 2013).

The relationship between heteroblasty and attainment of floral competence differs between species, and some species may even flower before vegetative changes become apparent (Poethig, 2010). Different signaling pathways may therefore exist (Matsoukas, 2014b; Hyun et al., 2017). Nevertheless, the juvenile-to-adult phase transition and attainment of floral competence are both controlled by decreased expression of miR156 and increased expression of its targets, the group of Squamosa Promoter Binding Protein-Like (SBP/SPL) transcription factor genes (Wu and Poethig, 2006; Wu et al., 2009; Xu et al., 2016) (Fig.1).

The juvenile-to-adult phase transition is dependent on shoot-derived factors associated with photosynthesis (Poethig, 2013). A role of sugar supply in the heteroblastic transition was demonstrated in research with the waterfern Marsilea (Allsopp, 1954): Addition of sugar to the culture medium resulted in earlier formation of adult leaves, and this process could be reversed by transfer to a sugar-free medium. In Arabidopsis, vegetative phase change from juvenile to adult leaves is characterized by increased leaf complexity, including the formation of abaxial trichomes and serrated margins. This transition is delayed in defoliated plants and in photosynthetic mutants (Yang et al., 2013; Yu et al., 2013; Buendía-Monreal and Gillmor, 2017), supporting the view that sugar produced in photosynthesis is involved. Under low sugar availability, high miR156 abundance prevents the transition from juvenile to adult leaves, whereas sugar supply represses the transcription of MIR156 genes and promotes phase change (Yang et al., 2013; Yu et al., 2013). Yu et al. (2013) show that this regulatory effect is evolutionarily conserved as Suc supply does not only repress miR156 in Arabidopsis, but also in other flowering plants and in the moss Physcomitrella patens.

There is also evidence for an involvement of HXK1-dependent signaling in the transition, however not necessarily in the way that would be expected. If HXK1 were responsible for the sensing of Glc during the juvenile-to-adult phase transition, one would expect the HXK1 mutant, gin2-1, to have an extended juvenile phase as it cannot respond to hexose accumulation. However, Yang et al. (2013) showed that gin2-1 produces fewer juvenile leaves and has lower levels of miR156 than wild-type plants, consistent with accelerated phase transition, although the differences were not very large. Matsoukas et al. (2013) determined the length of the juvenile phase as insensitivity to flowering induction upon transfer to long days and also observed that gin mutants, including gin2-1, had a shorter juvenile phase. The observation that mutants in starch...
synthesis, as well as mutants in starch breakdown, show an extended juvenile phase length demonstrates the complexity of carbohydrate signaling (Matsoukas et al., 2013). Since mutants in starch synthesis and breakdown have lower night-time Suc contents than wild-type plants, starvation during the night may be responsible for the delay in the juvenile-to-adult phase transition, but what the exact signal is and how it is sensed is not entirely clear.

A model for the potential involvement of SnRK1 and T6P in the juvenile-to-adult phase transition and flowering was developed by Tsai and Gazzarrini (2014). It was shown that T6P interacts with miR156 signaling during the floral transition (see below), but how SnRK1 and T6P may interact with miR156 in the vegetative (heteroblastic) transition remains an open question.

FLORAL TRANSITION

Under inductive conditions, the juvenile-to-adult phase transition is followed by the floral transition (vegetative-to-reproductive phase transition). It is not always possible to differentiate the processes during which plants acquire the competence to flower as part of the juvenile-to-adult phase transition from those responsible in floral transition as such, and the main function of sugars may be in regulating the competence to flower (Hyun et al., 2017). Accordingly, the regulatory framework of the juvenile-to-adult phase transition and floral transition appears to be the same (Fig. 1), with miR156-dependent repression of SPL genes and increased miR172 expression also involved in the regulation of flowering (Matsoukas, 2014b; Yu et al., 2015). While there is overlap in SPL function, different members of the SPL transcription factor family also have specific developmental functions in vegetative phase change and the floral transition (Xu et al., 2016).

Sugar availability is an important signal in floral induction. It was e.g. observed that Suc concentration in leaf exudate increases in response to an inductive long day (Corbesier et al., 1998), and Suc supply can promote flowering of Arabidopsis plants even in the dark (Roldán et al., 1999). It was therefore discussed if Suc may act as florigen (Corbesier and Coupland, 2006). While Flowering Locus T (FT) protein has since been identified as a long-distance signal for floral induction (Corbesier et al., 2007), this does not exclude the possibility that additional
florigens, such as gibberellins and Suc, play an important role in modulating flowering time (King, 2012), e.g. via stimulating FT expression (King et al., 2008).

Work with transgenic plants has supported the view that flowering is regulated by sugar metabolism. Plants overexpressing the Suc biosynthetic enzyme Suc-phosphate synthase (SPS) flowered earlier and produced more flowers (Micallef et al., 1995; Baxter et al., 2003). Meristem-specific expression of the Suc-hydrolyzing enzyme cell wall invertase also led to accelerated flowering and increased seed yield, whereas expression of cytosolic invertase had the opposite effect (Heyer et al., 2004). Mutants in starch synthesis and breakdown flower late (Corbesier et al., 1998), in addition to having an extended juvenile phase (Matsoukas et al., 2013). These findings support a role for Suc or the Suc/hexose ratio in flowering regulation. However, the response to Suc is not straightforward, as demonstrated by a delay of flowering at high (5%) external Suc concentration (Ohto et al., 2001).

Several lines of evidence support the view that T6P is required as a sugar signal in the regulation of flowering: Plants with lower T6P through expression of the *E. coli* TPP or TPH (trehalose-6-phosphate hydrolase) genes for T6P breakdown flower late (Schluepmann et al., 2003). Mutants in the Arabidopsis T6P synthase gene (*tps1* mutants) are embryo lethal, but they can be rescued by expression of *TPS1* from a dexamethasone inducible promoter (van Dijken et al., 2004) or a seed-specific promoter (Gómez et al, 2010) to investigate the effect of a lack of *TPS1* expression after germination. In both cases, growth was strongly reduced and no flowering occurred in the absence of *TPS1* expression, while low *TPS1* expression after dexamethasone treatment or in plants with weak *TPS1* alleles resulted in delayed flowering compared to wild-type plants. More recent work shed light on how T6P regulates flowering. Repression of *TPS1* through the use of artificial microRNA showed that T6P synthesis is required for the expression of FT (Wahl et al., 2013). In the shoot apical meristem, T6P is involved in the down-regulation of miR156 expression which is required for flowering, whereas in the leaves T6P acts directly by regulating FT expression (Fig. 1). In addition to the role of T6P in floral transition, a function of TOR in promoting flowering has been suggested (Ren et al., 2012).

Overexpression of the SnRK1 gene *AKIN10* leads to delayed flowering (Baena-González et al., 2007; Tsai and Gazzarrini, 2012). SnRK1 phosphorylates the Indeterminate Domain (IDD) transcription factor IDD8, which results in its inactivation and delayed flowering under sugar starvation (Jeong et al., 2015). While SnRK1 and IDD8 have opposite functions in the regulation of flowering in response to carbon availability,
overlapping functions between SnRK1 and its target FUS3 (which is stabilized after phosphorylation by SnRK1) have been described (Tsai and Gazzarrini, 2012). The findings discussed here are consistent with signaling pathways for high carbon availability inducing and SnRK1 repressing the floral transition.

SHOOT BRANCHING

Shoot branching results from the release of axillary bud dormancy. The classical view is that shoot branching is inhibited by auxin transport from the shoot apex to axillary buds (resulting in apical dominance), and that auxin depletion upon removal of the shoot tip is responsible for increased shoot branching. However, Mason et al. (2014) proposed that auxin depletion is not sufficient to trigger bud release. Instead, they show that, upon removal of pea shoot tips, more sucrose is transported to the axillary buds where it reduces expression of the branching inhibitor Branched1 (BRC1) gene. This effect is dependent on the presence of source leaves, supporting the notion that sink-source relationships determine shoot branching (Mason et al., 2014).

Meristem-specific overexpression of cell wall or cytosolic invertase in Arabidopsis changes the shoot branching pattern in a complex manner, differentially affecting the formation of axillary inflorescences, branching of the main inflorescence, and branching of side inflorescences (Heyer et al., 2004). This suggests that the sucrose/hexose ratio in specific cellular compartments is critical for the branching pattern.

The sucrose signal may be mediated by T6P signaling (Barbier et al., 2015). Apical dominance is enhanced in plants with lower T6P due to expression of the E. coli TPP or TPH genes, whereas plants expressing the E. coli TPS gene show increased branching, suggesting a role of T6P as a sugar signal in branching control (Schluepmann et al., 2003; van Dijken et al., 2004). The observation that the maize Ramosa3 gene, which regulates tassel and ear branching, encodes a TPP enzyme supports a function of T6P in inflorescence branching (Satoh-Nagasawa et al., 2006). A similar role was recently suggested for the grape Sister of Ramosa3 (VvSRA) (Ishiai et al., 2016), although a direct involvement in T6P
breakdown was not demonstrated. Eveland and Jackson (2012) point out that the maize Ramosa3 may also act as a transcriptional regulator and that its function may therefore not solely depend on its TPP catalytic activity.

In addition to T6P, HXK1 is involved in increased branching, as suggested by a loss of apical dominance in transgenic HXK1-overexpressing Arabidopsis plants (Kelly et al., 2012). Mutations in genes encoding proteins that interact with TOR also suggest a function of TOR-dependent signaling in the regulation of apical dominance and shoot branching: mutants in Raptor (Anderson et al., 2005) or LST8 (Moreau et al., 2012) have increased shoot branching, suggesting that the TOR pathway may inhibit shoot branching, although how this may affect the response to sugar availability has not been investigated.

Overall, there is strong evidence for a key role of sugars in the branching of vegetative shoots and inflorescences, but to what extent they are simply fuelling growth, act as metabolic regulators or interact with other developmental pathways is not always clear. In addition, miR156 overexpression enhances shoot branching in Arabidopsis (Schwarz et al., 2008; Wei et al., 2012) and Brachypodium distachyon (An et al., 2015). Whether or not this regulation is related to sugar signaling is, however, unknown.

SENESCENCE

During leaf senescence, nutrients are recycled from the old leaves. For example, nitrogen from photosynthetic proteins is exported and can be invested in the photosynthetic apparatus of younger organs or stored in seed storage proteins. Photosynthesis declines during this process, but, in the absence of strong sinks, old leaves do not become carbon starved and sugars can accumulate (Wingler et al., 2006). Senescence can be induced by dark treatment which results in starvation, but global changes in gene expression during dark treatment only show little similarity with developmental senescence (Wingler et al., 2009). Instead, senescence triggered by a combination of low nitrogen with 2% Glc supply (Wingler et al., 2004; Pourtau et al., 2004; Pourtau et al., 2006) shows high similarity with developmental senescence (Wingler and Roitsch, 2008; Wingler et al., 2009). Sugar accumulation in old leaves signals an excess of carbon relative to nitrogen availability, and sugars can thereby integrate other environmental signals to regulate nitrogen allocation (Wingler et al., 2006). Glc treatment only induces senescence from a certain
stage of development onwards (Wingler et al., 2012). This is in agreement with gene expression studies showing that sugar accumulation predominantly represses photosynthetic genes in older plants (unpublished comparison of microarray data by Price et al., 2004; Lloyd and Zakhleniuk, 2004; and Pourtau et al., 2006). A developmental time window for senescence regulation was proposed by Jing et al. (2002) who show that senescence can only be induced by ethylene during a defined period, suggesting that developmental factors are required to gain the competence to senesce.

Feeding of the metabolizable sugars Glc, Fru and Suc induces senescence to a similar extent (Wingler et al., 2012). However, a function of cell wall invertase activity in delaying leaf senescence in tobacco (Lara et al., 2004) and tomato (Jin et al., 2009) suggests that the Suc/hexose ratio in the apoplast is responsible, probably by influencing sink activity in the old leaves. Within the cells, HXK1-dependent signaling is involved in senescence regulation. Transgenic tomato (Dai et al., 1999; Swartzberg et al., 2011) and Arabidopsis (Kelly et al., 2012) plants overexpressing the Arabidopsis HXK1 have accelerated senescence, whereas the HXK1 mutant, gin2-1, shows delayed senescence (Moore et al., 2003) and a reduced senescence response to Glc treatment (Pourtau et al., 2006). While these findings support a role of HXK1 signaling in senescence regulation, a lack of HXK1 does not completely abolish the effect of sugar treatment on senescence, suggesting that other signaling pathways are involved.

In addition to HXK1, T6P is involved in senescence regulation in response to sugar availability. T6P accumulates during senescence in parallel with an increase in sugar contents (Wingler et al., 2012), and transgenic plants expressing a bacterial TPP gene to lower T6P content exhibit delayed senescence, as indicated by delayed leaf yellowing, decreased expression of senescence-associated genes and a lack of anthocyanin accumulation (Wingler et al., 2012). In addition, these plants do not show the typical senescence response to sugar treatment, suggesting that T6P is required for the induction of senescence by sugar availability. Transfer between different media with and without sugar showed that T6P only acts during a certain time window, after which sugar treatment also induces senescence in plants with low T6P (Wingler et al., 2012). This indicates that senescence induction at a later developmental stage is no longer T6P dependent. SnRK1 delays senescence (Baena González et al., 2007; Tsai and Gazzarrini, 2012), which is consistent with inhibition of SnRK1 by T6P (Zhang et al., 2009). However, inhibition of SnRK1 by T6P requires an additional factor that is present in growing tissues but not in senescing leaves (Wingler et al., 2012). If T6P acts
via SnRK1, it would therefore have to initiate senescence before the symptoms become visible, which is supported by the notion that T6P is required for early developmental changes that result in the competence to respond to other senescence-inducing factors, such as Glc or ethylene.

Inhibition of TOR delays senescence and flowering, which supports the notion that high energy availability promotes senescence (Ren et al., 2012). Since the timing of flowering and senescence is related (Wingler et al., 2010; Wingler, 2011), accelerated developmental senescence in response to high carbon availability may be a consequence of earlier flowering.

SEED DEVELOPMENT

During embryo maturation, storage compounds are synthesized in preparation for desiccation and dormancy. This stage is therefore characterized by a strong sink activity, and carbon signaling processes that regulate this process have been well researched. Embryo development in mutants in the Arabidopsis TPS (tps1 mutants) is arrested at the torpedo stage, showing that T6P is required for embryo maturation, especially for cell division and cell wall synthesis (Eastmond et al., 2002; Gómez et al., 2006). Using an elegant chemical approach, it was recently shown that T6P can directly promote grain filling in wheat. Spraying of wheat plants with compounds that release T6P upon exposure to sunlight led to increased grain yield due to the formation of larger grains with higher starch content (Griffiths et al. 2016b). Earlier work had shown that T6P naturally accumulates in wheat grains to previously unreported levels before grain filling (Martínez-Barajas et al., 2011). Interestingly, T6P content was low in the embryonic and maternal tissues and accumulated almost exclusively in the endosperm, although, at later stages, it may also be required in the embryo itself. In addition to T6P, TOR is required for endosperm and embryo development (Menand et al., 2002).

SnRK1 plays an important role in pea seed development: Antisense repression of SnRK1 in seeds resulted in defects in seed maturation and storage protein synthesis via ABA synthesis and/or signaling (Radchuk et al., 2006; 2010). SnRK1 has been proposed to regulate seed maturation by interacting with FUS3 and hormone signaling pathways (Tsai and Gazzarrini, 2012). SnRK1-dependent phosphorylation stabilizes FUS3, which is required for maturation by positively regulating ABA synthesis (Tsai and Gazzarrini, 2012, 2014). SnRK1 thus has a positive
effect on seed maturation and storage protein synthesis which is opposite to its characteristic role in starvation signaling and activation of catabolic pathways (Baena-González and Sheen, 2008). In developing wheat grains, in vitro SnRK1 activity was reported in the embryo and endosperm, which together with low T6P in the embryo, may support high in vivo SnRK1 activity for embryo maturation (Martínez-Barajas et al., 2011). The C/S1 bZIP transcription factors, in particular bZIP53 which is expressed late during seed development, have also been proposed to be involved in storage protein synthesis and seed maturation (Weltmeier et al., 2009; Alonso et al., 2009; Restovic et al., 2017).

Signaling pathways associated with high carbon availability (T6P and TOR) as well as those associated with starvation (SnRK1, C/S1-group bZIPs) are thus required for seed development. This may reflect different requirements in different tissues that act as source or sink (i.e. T6P in the endosperm and SnRK1 in the embryo), or different temporal patterns (T6P accumulating pre-grain filling and bZIP53 being expressed during maturation), but how exactly the various signaling pathways are coordinated is a question for future research.

CONCLUDING REMARKS

Developmental transitions that are associated with the creation of new sinks are usually triggered by high carbon signaling pathways, including T6P, hexokinase and TOR signaling, supporting the view that these transition can only go ahead when sufficient carbon is available (Table 1). While the involvement of sugar signaling pathways in seed germination, flowering and senescence is well investigated, more questions remain concerning the juvenile-to-adult phase transition, shoot branching and seed development (Outstanding Questions Box). Although the function of miR156 in the juvenile-to-adult phase transition has been explored in detail, interactions with sugar signaling are not always clear. For example, the function of T6P and SnRK1 in heteroblastic transitions still needs to be investigated, while information on the role of C/S2 bZIPs in developmental transitions is generally scant. For transitions that require complex changes in different tissues and cells of an organ (e.g. seed development or branching) it is necessary to investigate signaling processes at high temporal and spatial resolution e.g. using biosensors (Jones et al., 2013) and single cell analysis of metabolites, transcripts and protein after laser micro-dissection (Misra et al., 2014). Given that developmental transitions are often linked, developmental changes triggered by sugars early in development may have later consequences (e.g.
the juvenile-to-adult phase transition affecting floral transition, flowering affecting senescence, and shoot branching affecting senescence or vice versa), making it difficult to identify which process specifically is regulated by a signaling pathway. To elucidate these relationships requires developmentally timed manipulation of sugar signaling pathways, e.g. using inducible promoters.

**FIGURE LEGEND**

**Fig. 1.** Model for the role of sugar signaling during the juvenile-to-adult phase transition and floral transition, combining elements of the models presented by Tsai and Gazzarrini (2014) and Yu et al. (2015), in addition to highlighting the function of T6P in leaves and the shoot apical meristem (SAM), and the potential functions of HXK1 and TOR. T6P and SnRK1 may play a role in the juvenile-to-adult phase transition, in addition to flowering, but their role in this transition is not well understood. Red components are involved in high carbon signaling, blue components in starvation signaling.
Table 1. Involvement of main components of sugar signaling in developmental transitions. Plus signs (+) indicate acceleration of the process by the signaling component, minus signs (−) delay. Empty cells indicate lack of information, question marks (?) that there is no strong support for an effect.

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<td>Floral transition</td>
<td>source-to-sink</td>
<td>acceleration</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>Shoot branching</td>
<td>source-to-sink</td>
<td>acceleration</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td></td>
<td>+ (sugar signaling?)</td>
<td></td>
</tr>
<tr>
<td>Senescence</td>
<td>decreased source</td>
<td>acceleration (or delay during dark treatment)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed development</td>
<td>source-to-sink</td>
<td>acceleration</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance.
Matsoukas IG (2014a) Interplay between sugar and hormone signaling pathways modulate floral signal transduction. Frontiers in Genetics 5

Matsoukas IG (2014b) Attainment of reproductive competence, phase transition, and quantification of juvenility in mutant genetic screens. Frontiers in Plant Science 5


Tsai AYL, Gazzarrini S (2012) AKIN10 and FUSCA3 interact to control lateral organ development and phase transitions in Arabidopsis. Plant Journal 69: 809-821


Wingler A, Delatte TL, O'Hara LE, Primavesi LF, Jhurreea D, Paul MJ, Schluepmann H (2012) Trehalose 6-phosphate is required for the
onset of leaf senescence associated with high carbon availability. Plant Physiology 158: 1241-1251


Sugar signalling is involved in anaerobic germination tolerance, which allows successful seeding establishment under submergence. A rice trehalose-6-phosphate phosphatase gene (TPP7) was identified as determinant of a QTL for anaerobic germination tolerance. Presence of functional TPP enhances starch mobilization and coleoptile elongation.

Sugars promote the juvenile-to-adult phase transition via downregulation of the microRNA miR156. This process requires normal starch metabolism and is influenced by hexokinase-1.

A direct effect of trehalose-6-phosphate (T6P) on wheat grain development was shown by spraying plants with caged T6P precursors that release T6P upon exposure to sunlight. Spraying of these compounds increased grain size and grain yield per plant. In addition, recovery of growth after drought treatment was enhanced, demonstrating improved crop resilience.
OUTSTANDING QUESTIONS

• Are heteroblastic transitions and the attainment of floral competence during the juvenile-to-adult phase transition regulated by exactly the same pathways, or are different signals involved?

• How do SnRK1 and T6P affect the juvenile-to-adult phase transition, and do they interact with miR156?

• To what extent do sugars act as signals in shoot branching instead of simply fuelling growth?

• Is the function of miR156 in shoot branching related to sugar signalling, e.g. by T6P-dependent down-regulation of miR156 expression?

• How is TOR involved in shoot branching in response to sugar availability, and does it inhibit or enhance branching?

• To what extent are transitions later in development a consequence of sugar effects on other transitions that occur earlier?

• How are different signalling pathways during seed development (involving T6P, TOR, SnRK1, and bZIP) coordinated?
**BOX 1. Developmental Transitions Covered in This Update**

- **Germination and cotyledon greening:** both processes are discussed together as they are both inhibited by high external sugar supply, but there are also differences in their regulation.

- **Juvenile-to-adult phase transition:** includes heteroblastic transitions (e.g. changes in leaf morphology, vegetative phase change) as well as attainment of the competence to flower; refers to processes during the vegetative phase.

- **Floral transition (vegetative-to-reproductive phase transition):** follows the juvenile-to-adult phase transition and results in flowering.

- **Shoot branching:** requires bud release and results in reduced apical dominance; vegetative branching and branching of the inflorescence are covered together here.

- **Senescence:** results in the death of an organ and is associated with nutrient remobilization; only senescence of photosynthetic organs (leaves, green inflorescences, and green fruits) is discussed here.

- **Seed development:** endosperm formation and embryo development are covered here, but the focus is on the maturation phase during which storage compounds accumulate in the embryo.
BOX 2. Main Sugar Signalling
Pathways/Components in Plants Discussed in This Update

A. High carbon
Glc signalling by hexokinase-1 (HXK1)
Suc signalling by trehalose-6-phosphate (T6P)
High energy (Glc) signalling by target of rampamycin (TOR)

B. Low carbon
Starvation signalling by Snf1-related protein kinase-1 (SnRK1)
Starvation signalling by C/S1 bZIP transcription factors
Low sugar (Glc?) signalling by microRNA156 (miR156)


Cho YH, Sheen J, Yoo SD (2010) Low glucose uncouples hexokinase1-dependent sugar signaling from stress and defense hormone
abscisic acid and C2H4 responses in Arabidopsis. Plant Physiology 152: 1180-1182


SYNTHASE 1 mutant is associated with altered cell wall structure, decreased cell division and starch accumulation. Plant Journal 46: 69-84


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complex regulation of SPL15 in a miR156-controlled gene network. BMC Plant Biology 12


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