Substantial roles of hexokinase and fructokinase in the effects of sugars on plant physiology and development

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Abstract

The basic requirements for plant growth are light, CO\textsubscript{2}, water, and minerals. However, the absorption and utilization of each of these requires investment on the part of the plant. The primary products of plants are sugars, and the hexose sugars glucose and fructose are the raw material for most of the metabolic pathways and organic matter in plants. To be metabolized, hexose sugars must first be phosphorylated. Only two families of enzymes capable of catalysing the essential irreversible phosphorylation of glucose and fructose have been identified in plants, hexokinases (HXKs) and fructokinases (FRKs). These hexose-phosphorylating enzymes appear to coordinate sugar production with the abilities to absorb light, CO\textsubscript{2}, water, and minerals. This review describes the long- and short-term effects mediated by HXK and FRK in various tissues, as well as the role of these enzymes in the coordination of sugar production with the absorption of light, CO\textsubscript{2}, water, and minerals.

Key words: Fructokinase, guard cells, hexokinase, hexose phosphorylation, minerals, photosynthesis, stomata, sugar, transpiration, water.

Sugars integrate and affect resource consumption and govern plant development and physiology

Plants need light, CO\textsubscript{2}, water, and minerals (such as ammonium, nitrate, phosphate, potassium, and several other essential elements) to grow. However, the absorption and utilization of each of these requires investment on the part of the plant. For example, investment in leaf formation and plastids is necessary for the absorption of light and the incorporation of CO\textsubscript{2}, while investment in roots and vascular tissues is necessary for the uptake and transport of water and minerals. Even under optimal conditions, when all resources are present in sufficient amounts, the absorption of each input should be well coordinated with that of the others, to ensure optimal investment in development and activity of specialized tissues and plant parts. The sessile nature of plants, which inevitably increases the unpredictability of resource availability, further necessitates such coordination.

Among the essential resources, only light may be considered relatively predictable. The availability of the other resources—CO\textsubscript{2}, water, and minerals—is much less predictable. In fact, the consumption of CO\textsubscript{2} and minerals is highly dependent on the presence of water, which is usually the major limiting factor for terrestrial plants. For this reason, terrestrial plants are coated with a water-impermeable cuticle layer that prevents evaporation and water loss (Edwards, 1993). Special pores, known as stomata, allow the uptake of CO\textsubscript{2} for photosynthesis during the day, but under water-limiting conditions, the stomata are closed. As a result, the ability of CO\textsubscript{2} to enter the plant is dependent on the presence of water, which causes the stomata to open to allow the uptake and assimilation of CO\textsubscript{2} (Raven, 2002). Similarly,
the acquisition of minerals from the soil and the transport of minerals within the plant are also dependent on water (Smith, 1991). In this manner, water governs the unpredictable nature of the resources required for plant survival, growth, and reproduction. Once water becomes available, plants respond rapidly, opening stomata to absorb CO₂ and (indirectly) minerals necessary for the production of sugars. Water is released to the atmosphere through the open stomata, creating a vacuum that draws additional water and minerals into the plant from the soil solution.

Sugars are the primary products of plants and are the raw material for all of the metabolic pathways and organic matter in plants. Accordingly, sugar allocation determines the plant’s ability to absorb resources and grow. For example, when there is a shortage of water or minerals, more of the available sugar is diverted towards root development and the improvement of root absorption functions, at the expense of aboveground vegetative tissues (Smith, 1982). On the other hand, when light is in limited supply, more of the available sugar will be directed towards stem elongation and the development of leaves and plastids (Poorter and Nagel, 2000). Therefore, it is reasonable to assume that sugars might coordinate the absorption and uptake of light, CO₂, water, and minerals with plant physiology and development.

### Short- and long-term effects of sugars and molecular pathways that mediate these effects

The effects of sugars can be roughly divided into short- and long-term responses. Short-term responses include rapid, reversible physiological effects such as changes in stomatal aperture, photosynthesis rate, starch accumulation, and the absorption of minerals by the roots. Long-term responses might include slow, long-lasting, possibly irreversible developmental changes such as shoot formation, leaf development, leaf senescence, and root and vascular development. Several molecular mechanisms that mediate sugar responses have been identified in plants (reviewed by Smeekens et al., 2010). These mechanisms play roles in two types of situation: when excess sugar is present and when sugar is in limited supply. The hexokinase (HXK) sugar-sensing pathway, the trehalose-6-phosphate (T6P) pathway, and target of the rapamycin (TOR) kinase pathway all play roles in the presence of excess sugar, whereas SNF-related protein kinases (SNRKs) and the C/S1 bZIP transcription factor network play important roles when sugar is in limited supply (Smeekens et al., 2010). The fructokinases/sucrose synthase (FRK/SUS) pathway may also control sugar allocation in response to sugar levels (Granot et al., 2013). This review will focus on the roles of the hexose-phosphorylating enzymes, HXK and FRK, in the short- and long-term effects of sugars on plant physiology and development, and will also describe how HXK and FRK coordinate plant physiology and development with resource availability.

### Origin of the sugars found in source and sink tissues and their localization

The production of sugars by photosynthesis has been widely described and has been illustrated in recent reviews (Stitt et al., 2010; Granot et al., 2013). Nevertheless, it might be appropriate to review some of the details here, to emphasize the origin and distribution of glucose and fructose, the substrates of HXK and FRK.

Sucrose is the primary end product of photosynthesis (Fig. 1). This sucrose can then be metabolized in photosynthetic tissues, or exported out of the photosynthetic (source) tissues to non-photosynthetic (sink) tissues, where it serves as an initial substrate for all organic metabolic pathways (Fig. 1). To be metabolized in sink or photosynthetic source tissues, sucrose must be cleaved by either invertase (INV) or sucrose synthase (SUS), the only two families of sucrose-cleaving enzyme that have been identified in plants (Dennis and Blakeley, 2000). INV cleaves sucrose into the monomer hexoses glucose and fructose, whereas SUS cleaves sucrose in the presence of UDP to yield UDP-glucose (UDP-G) and fructose (Fig. 1). Starch in photosynthetic tissues (during the dark period) and in sink tissues is also degraded to yield glucose monomers (Chia et al., 2004; Smith et al., 2005). Both hexoses, glucose and fructose, must be phosphorylated before they can be used in any metabolic process. Hence, hexose-phosphorylating enzymes are essential for all aspects of plant metabolism and development. While the metabolic function of hexose kinases in sink tissues is essential, their function in photosynthetic tissues during the day may seem dispensable, as the hexoses obtained from triose phosphates are already phosphorylated (Fig. 1). Nevertheless, hexose kinases are present in source tissues (Claeyssen and Rivoal, 2007; Granot, 2008; Granot et al., 2013) and play a crucial role in sugar sensing, as described below.

### Plant hexokinases and fructokinases

Only two types of glucose- and fructose-phosphorylating enzymes have been discovered in plants, HXKs and FRKs. As such, HXKs and FRKs are the gateway for most of the organic metabolism in plants. Moreover, these enzymes catalyze irreversible reactions (no hexose-phosphate phosphatases have been found in plants) and therefore may play central roles in the regulation of plant sugar metabolism. While FRK activity is specific to fructose, HXKs are capable of phosphorylating glucose, fructose, mannose, and glucosamine (reviewed by Granot, 2007, 2008). Accordingly, in plants, glucose can be phosphorylated only by HXK, whereas fructose can be phosphorylated by either HXK or FRK. Yet, the affinity of HXKs for glucose is in the 0.02–0.1 mM range, whereas their affinity for fructose is about one to three orders of magnitude lower, in the 2–120 mM range (Granot, 2007). The affinity of FRKs for fructose is usually high, within the same range as the affinity of HXKs for glucose (Pego and Smeekens, 2000; Granot, 2007). As the affinity of HXKs for glucose is much higher than their affinity for fructose, it has been suggested that in vivo HXKs probably phosphorylate mainly glucose, whereas fructose is phosphorylated primarily by FRKs (Granot, 2007). Several
FRK isozymes are inhibited by their own substrate, fructose, when it exceeds a certain concentration. This phenomenon is known as substrate inhibition and its putative biological significance is described below (Pego and Smeekens, 2000; German et al., 2003; Damari-Weissler et al., 2009).

So far, three to ten HXK genes and three to seven FRK genes have been found in different plant species (Cho et al., 2006a; Granot, 2007; Karve et al., 2010). Two major types of HXK have been identified in plants based on their intracellular localization, which is determined by their N-terminal amino acid sequences (Olsson et al., 2003; Giese et al., 2005; Cho et al., 2006a; Damari-Weissler et al., 2006; Kandel-Kfir et al., 2006; Karve et al., 2008). Type A HXKs are located within plastids and type B HXKs are associated with the mitochondria. A third type, type C HXK, is located in the cytoplasm of monocots (da-Silva et al., 2001; Cho et al., 2006a; Karve et al., 2010; Cheng et al., 2011). Similar to HXKs, FRKs are found in two locations in plants, in plastids and in the cytoplasm. Most plant species have a single plastidic HXK and a single plastidic FRK, with multiple HXK and FRK isozymes in the cytoplasm. Yet, while the cytoplasmic FRKs are located within the cytosol, all of the cytoplasmic HXKs in eudicots and most of the cytoplasmic HXKs in monocots are associated with the mitochondria (Granot, 2008; Granot et al., 2013).

The HXK sugar-sensing pathway

As HXKs are the only enzymes that can phosphorylate glucose in plants, they are probably present in most, if not all types of plant cells (Granot et al., 2013). Over the last two
and a half decades, the role of HXK in plants has been studied by exposing plant cells and seedlings to HXK substrates and substrate analogues and by overexpressing and downregulating type B HXK in planta. The Arabidopsis type B HXK (AtHXK1) was identified as a genuine glucose sensor that mediates glucose effects independently of its glucose phosphorylation enzymatic activity [i.e. independently of its products, glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P), and their downstream metabolism] (Jang and Sheen, 1994; Jang et al., 1997; Moore et al., 2003). Overexpression of AtHXK1, either the native or the mutated isoform that lacks the hexas phosphorylation catalytic activity, inhibits the expression of photosynthetic genes, decreases chlorophyll levels and reduces the rate of photosynthesis (Jang et al., 1997; Dai et al., 1999; Xiao et al., 2000; Moore et al., 2003; Kelly et al., 2012). These effects suggest that AtHXK1 monitors glucose levels, most likely in mesophyll cells of photosynthetic tissues, and inhibits photosynthesis when glucose levels are sufficiently high (Jang et al., 1997; Dai et al., 1999; Moore et al., 2003; Kelly et al., 2012). This role of HXK in shoots (photosynthetic plant parts) as compared with roots was confirmed by reciprocal grafting experiments in which the effects of overexpression of AtHXK1 were observed only when this overexpression occurred in the shoots (Dai et al., 1999).

The regulation of photosynthesis by HXK may represent a short-term, reversible sugar-sensing effect aimed at balancing the investment in sugar production (such as expression of photosynthetic genes and chlorophyll production) with sugar levels and the capability of utilizing the sugars. Although constitutive expression of AtHXK1 in Arabidopsis and tomato plants (under the 35S promoter) has a long-term growth-inhibiting effect (Jang et al., 1997; Dai et al., 1999; Kelly et al., 2012), that growth inhibition might be in part an artificial effect caused by the continuous unnatural reduction of photosynthesis by HXK.

The origin of glucose sensed by HXK in photosynthetic mesophyll cells during the day is not clear, as there is seemingly no need for production of free glucose in these tissues in the presence of light (Fig. 1) (Granot et al., 2013). Furthermore, unlike fungal and mammalian cells, there is no evidence for the production of glucose monomers via gluconeogenesis in plants, as no hexose-phosphate phosphatases have been found in plants. Rather, glucose monomers might be generated from starch degradation during the day, from the cleavage of intracellular sucrose by cytoplasmic invertase (cINV) and/or from the cleavage of some of the exported extracellular sucrose by cell wall invertase (cwINV) and subsequent importation via glucose transporters. Although the cleavage of trehalose (a glucose–glucose disaccharide) by trehalase yields glucose monomers that could potentially be sensed by HXK (Fig. 1), trehalose is usually found in very small quantities that may not be sufficient to stimulate a HXK sugar-sensing effect (Paul, 2007). Furthermore, the effects of exogenously added trehalose on plant growth and sugar metabolism occur independently of the expression level of AtHXK1 (Ramon et al., 2007), perhaps eliminating trehalose as a potential source of the glucose sensed by HXK in photosynthetic tissues. These results may also eliminate the possibility that G6P produced by HXK is important for the synthesis of T6P by trehalose phosphate synthase (TPS), the enzyme that generates T6P from UDP-G and G6P. Nevertheless, expression of Arabidopsis TPS1 might be dependent on AtHXK1 (Avonce et al., 2004), perhaps representing a common role in the short-term effects of HXK and T6P. Such induction of TPS1 expression by AtHXK1 (via an as-yet-unknown mechanism) may indicate the presence of excess sugar, which may further inhibit photosynthesis and divert resources from sugar production to starch accumulation.

It has been demonstrated that a small fraction of the mitochondria-associated AtHXK1 is transported to the nucleus, where it forms a complex that includes the vacuolar H+-ATPase B1 (VHA-B1) and the 19S regulatory particle of the proteasome subunit (RPT5B) (Cho et al., 2006b). This complex can bind the promoters of specific genes, such as CAB2, and may inhibit their expression independently of glucose metabolism, further supporting the role of HXK as a glucose sensor in photosynthetic tissues that mediates the short-term effects of sugar on photosynthesis.

Constitutive overexpression of AtHXK1 in tomato and Arabidopsis plants under the 35S promoter accelerates leaf senescence (Dai et al., 1999; Xiao et al., 2000; Swartzberg et al., 2011; Kelly et al., 2012). Leaf senescence is occasionally associated with high sugar levels and with the remobilization of nitrogen and minerals from senescing (old) leaves to other plant parts (Wingler et al., 2006; Wingler and Roitsch, 2008). It is very likely that, under normal conditions, high levels of sugar in old leaves are sensed by HXK and stimulate senescence. Such an effect would represent a long-term effect of HXK (Dai et al., 1999; Xiao et al., 2000; Moore et al., 2003).

In addition to its role as a glucose sensor in photosynthetic tissues that probably inhibits unnecessary investment in photosynthesis, HXK is also required for proper growth. For example, the Arabidopsis hxl1 (gin2-1) mutant displays reduced shoot and root growth under increasing light intensities (Moore et al., 2003). While HXK inhibition of photosynthesis is a genuine signalling effect that is independent of HXK catalytic activity (Moore et al., 2003), the importance of the signalling function of HXK in growth promotion as compared with that of its metabolic function is not yet clear. Suppression of the mitochondria-associated NhHXK1 in tobacco plants resulted in growth inhibition and in the accumulation of starch and glucose, suggesting that the NhHXK1 has a metabolic function and is essential for maintaining starch turnover, glycolytic activity, and normal growth (Kim et al., 2013).

A role for HXK in the juvenile-to-adult transition

After germination, plants undergo transitions between juvenile, adult, and reproductive developmental stages. The transitions between these stages are governed by various external and endogenous factors, such as day length, temperature, and nutritional status (Poethig, 1990; Amasino, 2010). It has been established that, after germination, microRNA156 (miR156)
function is necessary and sufficient for the transition to the juvenile stage, while its repression allows the transition from the juvenile stage to the adult stage (Wu et al., 2009). Recently, the repression of miR156 has been shown to be mediated by a leaf-derived signal (Yang et al., 2011). Furthermore, newly published studies investigating the connection between nutritional status and the juvenile-to-adult transition in Arabidopsis have demonstrated that the leaf-derived signal is sugar (Yang et al., 2013; Yu et al., 2013). It has been shown that chlorophyll-deficient mutants and plants from which leaves have been removed have elevated miR156 levels and demonstrate delayed juvenile-to-adult transition. In addition, external sugar application, which resulted in the downregulation of miR156, further demonstrated the role of photosynthesis products in phase transition. Analyses of the gin2-1 mutant at different developmental stages have revealed that the miR156 transcript level at the early juvenile developmental stage is partially dependent on HXK signalling, whereas the expression of miR156 at the later stage is independent of HXK (Yang et al., 2013; Yu et al., 2013). These results suggest that HXK prevents the accumulation of miR156 and promotes early juvenile phase transition in response to sugar. Additional studies are required to determine how sugar prevents the accumulation of miR156 at later developmental stages independently of HXK. Interestingly, suppression of miR156 is also required for the transition to the reproductive (floral) stage and the accumulation of miR156 is affected by TPS1 and T6P (Van Dijken et al., 2004; Wahl et al., 2013). However, the possible link between HXK and the floral phase has not yet been tested.

**HXK in the regulation of stomatal closure**

A surprising role for HXK in guard cells and stomatal behavior has recently been discovered (Kelly et al., 2013). About a century ago, it was proposed that sugars generated from the degradation of starch in guard cells at dawn are the primary guard-cell osmolytes stimulating the movement of water into the guard cells and stomatal opening (Lloyd, 1908). This hypothesis was later modified by the discovery that K⁺ ions, together with Cl⁻ and malate ions, are the primary osmolytes that accumulate in guard cells and open stomata (Schroeder et al., 2001; Roelfsema and Hedrich, 2005; Pandey et al., 2007). However, in addition to these ions, it has been suggested that the accumulation of sucrose in guard cells over the course of the day as a result of starch degradation, photosynthetic carbon fixation, or the import of apoplastic (intercellular) sucrose also contributes to the osmotic state of the guard cells and the opening of the stomata (Gotow et al., 1988; Tallman and Zeiger, 1988; Poffenroth et al., 1992; Talbott and Zeiger, 1993, 1996).

The data from the studies that do support the role of sucrose as a stomata-opening osmolyte are mostly correlative. Sucrose levels in guard cells have been measured in correlation with stomatal aperture (Amodeo et al., 1996; Talbott and Zeiger, 1998). Otherwise, almost no functional studies of the role of sucrose in guard cells have been carried out (Talbott and Zeiger, 1988, 1998; Lawson, 2009). In contrast to the prevailing hypothesis, a recent study has shown that sucrose closes stomata (Kelly et al., 2013). Furthermore, the closure of stomata by sucrose is mediated by HXK and abscisic acid (ABA) within guard cells (Kelly et al., 2013). These findings support the existence of a feedback-inhibition mechanism that is mediated by sugars (the product of photosynthesis) and HXK (Outlaw, 2003; Kang et al., 2007). According to this hypothesis, when the rate of sucrose production exceeds the rate at which sucrose is loaded into the phloem, the surplus sucrose is carried towards the stomata by the transpiration stream and stimulates stomatal closure via HXK, thereby preventing the loss of precious water. This feedback system may represent a short-term, rapid mechanism that coordinates CO₂ uptake, sugar production, and sugar utilization with water loss. This mechanism inevitably also controls the uptake of water and minerals by the plant.

The effect of HXK on stomatal closure and its indirect effect on the uptake of water and minerals are in line with the effects of HXK on the mesophyll photosynthesis rate. The effect of HXK on photosynthesis in guard cells has not yet been studied. Yet, in both mesophyll and guard cells, excess sugar is sensed by HXK, leading to reduced expression of photosynthetic genes in mesophyll cells and closure of the stomata. The closure of the stomata reduces CO₂ uptake, further minimizing the rate of mesophyll photosynthesis. These HXK-mediated effects are probably part of a constant, ongoing physiological response that coordinates sugar production with the investment in photosynthesis and water uptake and water loss (Fig. 2). When the sugars are used and sugar levels carried towards the stomata drop, the expression of photosynthetic genes and the uptake of CO₂, water, and minerals may resume, once again allowing the production of sugars.

ABA appears to be essential for HXK-mediated effects. ABA mutants with mutations involving ABA biosynthesis genes such as *ABA1* (zeaxanthin epoxidase), *ABA2* (short-chain dehydrogenase reductase 1), and *ABA3* (molybdenum cofactor sulfurase), or in the ABA signalling pathway, such as the ABI3s (ABA-insensitive) 3–5 (B3, AP2, and bZIP transcription factors, respectively) are insensitive to HXK-mediated photosynthetic and growth-inhibiting effects (Zhou et al., 1998; Laby et al., 2000; Cheng et al., 2002; Leon and Sheen, 2003; Rolland et al., 2006; Rognoni et al., 2007; Ramon et al., 2008). ABA produced in guard cells is also necessary for the HXK-mediated stomatal closure (Kelly et al., 2013). Unlike the situation in guard cells, in which the molecular role of ABA in stomatal closure is well known, the molecular mechanism by which ABA participates in the inhibition of photosynthesis is not known. In all studies to date, inhibition of photosynthesis and growth has been observed upon the expression of HXK under the global 35S promoter, which is also expressed in guard cells. It is tempting to speculate that some portion of the observed photosynthetic and growth-inhibiting effects might be due to the stomatal closure effect of HXK in guard cells, which may provide an explanation for the involvement of ABA. Yet Arabidopsis and tomato plants that express HXK specifically in guard cells do not exhibit any inhibition of photosynthesis or growth (Kelly et al., 2013), perhaps indicating that ABA plays a guard-cell-independent role.
Fig. 2. Roles of HXK and FRK in plant physiology. During the day, HXK plays a continuous role in the coordination of sugar production with the uptake of CO$_2$, water, and minerals. This figure illustrates the direct inhibitory effects (solid lines) of HXK on photosynthesis and transpiration, and how stomatal closure by HXK reduces the uptake of CO$_2$, water, and minerals (dashed blue line). HXK also enhances mineral uptake by roots (dashed black line). Unlike HXK, FRK controls the allocation of sugar for vascular development, which may also affect sugar water and mineral transport and whole-plant development and physiology. N, nitrogen; P, phosphate; K, potassium; Zn, zinc; S, sulfur. (This figure is available in colour at JXB online.)

**A role for HXK in the uptake of minerals**

Photosynthesis and sugars have been shown to play a dominant role in regulating root mineral uptake (Forde, 2002a, b; Lillo, 2008). Several studies have shown that, upon high sugar production by photosynthesis or external application of sugar, the expression of different ion transporters that facilitate the transport of nitrate, ammonium, oligopeptides, sulfur, zinc, and potassium is induced in roots (Lejay et al., 2003, 2008). Nineteen ion transporter-encoding genes were found to be upregulated by sugars and/or upon light exposure (Lejay et al., 2008). Using the HXK inhibitor glucosamine, the authors of that study found that the expression of 16 of the 19 genes was HXK dependent. Yet, mannose, a HXK substrate that is believed to stimulate the HXK sugar-sensing pathway but is poorly further metabolized (Klein and Stitt, 1998; Pego et al., 1999), did not stimulate the expression of the above genes (except for the oligopeptide transporter At5g41000), suggesting that the HXK-dependent expression of the ion transporters is unrelated to sugar sensing but may be related to sugar metabolism downstream of HXK (Lejay et al., 2003, 2008). Yet, the nitrate transporters of maize (Zea mays), ZmNrt2.1 and ZmNrt2.2, have been shown to be upregulated by sugars and by sugar analogues known to stimulate the HXK sensing mechanism (Trevisan et al., 2008).

A role for HXK in mediating the crosstalk of sugars and phosphate uptake has also been proposed. The phosphate starvation response in *Arabidopsis*, which requires sugars, induces lateral root formation and expression of phosphate starvation-induced (PSI) genes. *AtHXK1 (gin2-1)* mutants display a significant reduction in lateral root formation and suppression of PSI genes (Karthikeyan et al., 2007). These results suggest that the interaction between sugars and phosphate is mediated by HXK but could be related to sugar metabolism downstream of HXK involving glycolysis rather than sugar sensing. A HXK-independent pathway for the interaction between sugars and the phosphate starvation response has also been proposed (Muller et al., 2005).

**FRKs and fructose sensing**

Fructose accounts for at least half of the hexose obtained from sucrose cleavage. As the affinities of FRKs for fructose are usually two orders of magnitude greater than those of HXKs for fructose (Granot, 2007), it was hypothesized that FRK, like HXK, might act as a fructose sensor. However, no role for FRK (or HXK) in fructose sensing has been established. Rather, growth inhibition of *Arabidopsis* seedlings in 6% fructose is mediated by fructose 1,6-bisphosphatase (FINS1/FBP) and the transcription factor FSQ6/ANAC089 (Cho and Yoo, 2011; Li et al., 2011). A truncated form of FSQ6/ANAC089 lacking the membrane-associated domain (thus rendering the FSQ6/ANAC089 transcription factor constitutively active) abolishes the growth-inhibition effect of fructose (Li et al., 2011). These results indicate the existence of a fructose-specific signalling pathway in *Arabidopsis* that does not involve FRK and HXK.

**A role for FRKs in vascular development**

It has been proposed previously that FRKs are important for starch accumulation in tomato (Schaffer and Petreikov, 1997b). However, studies of tomato and potato plants with...
Dai Morell and Damari-Weissler staining of German Beta vulgaris Plomion- 

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Involvement of FRK in other developmental and physiological processes

The tomato FRK4 is specifically expressed in anthers during the late stages of pollen development and during pollen germination (David-Schwartz et al., 2013), suggesting that this enzyme might be required for the procurement of the carbohydrates necessary for cell-wall synthesis during pollen-tube development. Tomato FRK1 was found to be related to flowering, as FRK1-antisense plants showed delayed transition to flowering (Odanaka et al., 2002). Other FRKs might be involved in responses of plants to abiotic stress. In maize, FRK2 is upregulated in response to short-term salt stress (Zorb et al., 2011). In sunflower (Helianthus annuus), a plastidic FRK is upregulated along with other proteins related to carbon metabolism, in response to drought stress (Fulda et al., 2011). In sugar beet (Beta vulgaris) roots, an increase in FRK activity was observed in response to wound stress (Klotz et al., 2006). In rice (Oryza sativa), two FRKs are differentially regulated under anoxic conditions: OsFK2 expression increases, while OsFK1 expression decreases under low-oxygen conditions (Guglielmietti et al., 2006). The differential expression patterns and different kinetic properties (primarily the substrate inhibition) of different FRKs suggest that different FRKs may play important roles in regulating the amount of carbohydrate metabolized in different tissues under changing conditions.

Summary

HXK and FRK are important for physiological and developmental processes at the whole-plant level, coordinating carbohydrate availability with growth. Ever-changing environmental conditions and nutrient availability necessitate rapid, short-term responses to coordinate sugar production with the availability of water and minerals. The mitochondria-associated HXK appears to act as a sugar sensor in the mesophyll and guard cells of photosynthetic tissues, for the rapid, short-term coordination of the rate of photosynthesis (sugar production) with the rate of transpiration. When the sugar level is high, HXK inhibits the expression of photosynthetic genes
and reduces the rate of photosynthesis and, at the same time, closes the stomata, thereby reducing the uptake of CO₂ (further reducing photosynthesis) and reducing transpiration and water loss (Fig. 2). In turn, reduced transpiration limits the uptake of water and minerals from the soil solution (Fig. 2).

In addition, HXK in roots mediates sugar stimulation of root mineral uptake required for the utilization of sugars and the production of other organic molecules, such as amino acids and nuclear acids. In this way, HXK coordinates sugar production with the absorption and uptake of light, CO₂, water, and minerals. The effects of HXK on photosynthesis, stomata, and mineral uptake suggest that HXK plays a continuous diurnal role in the coordination of sugar production with the absorption of light, CO₂, water, and minerals.

Unlike HXK, there is no evidence to date for the involvement of FRK in sugar sensing. Rather, specific FRKs may control the amount of sugars allocated for the development of vascular tissues. As such, FRKs affect long-term developmental processes important for the transport of sugar, water, and minerals. Together, it appears that (specific) HXK and FRK enzymes, the only two groups of enzymes catalysing the absorption and uptake of light, CO₂, water, and minerals, are involved in short- and long-term effects that coordinate sugar production with the absorption of light, CO₂, water, and minerals and determine whole-plant physiology and development.

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