The tomato cytosolic fructokinase FRK1 is important for phloem fiber development

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Abstract

Tomato (*Solanum lycopersicum*) plants have four fructokinase genes, *SlFRK1-4*. The *SlFRK4* is expressed only in pollen, whereas the other three are expressed in all plant parts. While *SlFRK2* and *SlFRK3* are involved in vascular tissue development and affects the shape, size, and cell-wall width of xylem vessels and xylem fibers, the role of *SlFRK1* has not been studied previously. The current work investigates the expression of *SlFRK1* using transgenic tomato plants expressing the β-glucuronidase reporter gene under the *SlFRK1* promoter, as well as the role of *SlFRK1* using transgenic plants with antisense suppression of *SlFRK1*. The *SlFRK1* promoter was expressed primarily in vascular tissues and specific suppression of *SlFRK1* reduced water transport in stems, but had no other anatomical or phenotypic effects. Combined suppression of *SlFRK1* and *SlFRK2* severely inhibited plant growth and an anatomical analysis revealed a reduction in secondary xylem area and distorted phloem fibers characterized by thin cell walls and reduced lignification. The results suggest that *SlFRK1* is involved in vascular tissue development and hydraulic conductivity in tomato plants and that *SlFRK1* is important for normal phloem fiber development together with *SlFRK2*.

Additional key words: cell walls, hydraulic conductivity, lignification, water transport, xylem vessels.

Introduction

Sucrose, a disaccharide, is an important end product of photosynthesis and a primary carbon source for metabolism in sink tissues of many plants including tomato (*Solanum lycopersicum*). Sucrose must be cleaved by sucrose synthase (SUS), into UDP-glucose and fructose, or by invertase into glucose and fructose to be further metabolized (Dennis and Blakeley 2000). The free hexoses, fructose and glucose, must then be phosphorylated by fructokinase (FRK) or hexokinase (HXK) before they can enter metabolic pathways. The FRK and HXK are distinguished by their substrate specificities and affinities (Renz and Stitt 1993, Dai *et al.* 2002, Granot 2007). The FRK phosphorylates only fructose; whereas HXK phosphorylates both glucose and

fructose. However, the affinity of FRK for fructose is two orders of magnitude greater than that of HXK to fructose. It is, therefore, likely that fructose is phosphorylated primarily by FRK (Granot 2007).

The *FRK* genes have been identified in many plant species including tomato, potato, maize, soybean, barley, spinach, and pea. In tomato, four *FRK* genes (*SlFRK1-4*) have been identified. They encode enzymes with different intracellular localization and biochemical characteristics. The tomato *FRKs* are expressed at different levels in almost all plant parts with the exception of *SlFRK4*, which is expressed specifically in stamens and pollen (German *et al.* 2002, 2003, 2004, David-Schwartz *et al.* 2013, Granot *et al.* 2013). Three of the tomato FRK

enzymes are found in the cytosol and a single FRK (*SlFRK3*) is found in plastids (Damari-Weissler *et al.* 2006).

Some FRK enzymes are inhibited by their own substrate, fructose, when the fructose concentration exceeds a certain level, usually 1 mM, a phenomenon known as substrate inhibition (Gardner *et al.* 1992, Renz and Stitt 1993, Dai *et al.* 1997). In tomato, two FRK isozymes, SlFRK2 and SlFRK3, are substrate inhibited, whereas SlFRK1 and SlFRK4 are not (Petreikov *et al.* 2001, German *et al.* 2002, 2004). In addition, the affinity of SlFRK1 for fructose is about 20-fold less than that of SlFRK2 and SlFRK3 and only 2- to 8-fold greater than the affinities of the four tomato HXKs, SlHXK1-4 (Granot 2007).

The different characteristics of the various FRKs suggest that they may play different roles. Increasing evidence suggests that FRKs are important for vascular development. The tomato *SlFRK2* is essential for proper vascular development as stems of *SlFRK2*-antisense plants have xylem vessels and xylem fibers with thinner secondary cell walls and the xylem vessels of those plants are also narrower and deformed (Damari-Weissler *et al.* 2009). As a result, water transport is reduced, causing severe growth inhibition and the wilting of young leaves (Damari-Weissler *et al.* 2009). The tomato plastidic *FRK*, *SlFRK3*, is also important for xylem development. RNAi

suppression of *SlFRK3* decreased plant hydraulic conductivity and transpiration, but had no visual effect on plant growth. Yet, combined suppression of both the cytosolic *SlFRK2* and the plastidic *SlFRK3* disturbed not only xylem vessels, but also the development of xylem fibers, resulting in unlignified and distorted xylem fibers (Stein *et al.* 2016). FRK is also important for the development of xylem fibers in aspen (*Populus tremula* × *tremuloides*), in which the suppression of the cytosolic *FRK2* yielded narrower xylem fibers probably due to decrease in cellulose deposition (Roach *et al.* 2012). FRKs have also been found to be important for vascular tissue development in *Arabidopsis*. *Arabidopsis* plants harboring mutations in several FRKs (together) showed reduced xylem area and necrotic lesions in their xylem, cambium and the phloem of their hypocotyls, which led to reduced hydraulic conductivity and wilting of leaves and, eventually to plant death (Stein *et al.* 2017).

Of the three tomato FRKs expressed in various tissues, the role of *SlFRK1* has been the least studied. In a previous study, suppression of *SlFRK1* delayed flowering with no other effects on plant growth (Odanaka *et al.* 2002). The aim of this work was to explore the expression pattern and function of *SlFRK1* and to examine whether this gene also plays a role in vascular development.

Materials and methods

Plants and sequencing: Tomato (*Solanum lycopersicum* Mill.) plants of cultivar MP1 were used to generate all of the transgenic lines. The DNA of the BAC-167A6 was used for PCR amplification of the *SlFRK1* promoter region. A 1673-bp fragment of the *SlFRK1* promoter was amplified with a pair of primers carrying *EcoRI* and a *BamHI* restriction sites. After digestion, the DNA fragment was inserted into the pE234 plant binary vector, which had been predigested with *EcoRI* and a *BamHI*. The pE234 includes a *GUS* reporter gene and the neomycin phosphotransferase II (*NptII*) gene as a selectable marker.

The partial sequence between 880 and the 3' end of the FRK1 cDNA (Kanayama *et al.* 1997) was amplified using PCR. The primer contained an engineered SacI site (5'-CTAGCGAGCTCGAGTGGAATAATGAG-3'). The resulting 700-bp fragment was digested with SacI and BamHI (+1580). Each fragment was inserted between the cauliflower mosaic virus 35S promoter and nopaline-synthase termination site in the binary vector pBI121 (*Clontech*, Palo Alto, CA, USA), in which the GUS sequence was removed by digesting with BamHI (Odanaka *et al.* 2002). Both constructs were transformed into cv. MP-1 *via Agrobacterium*-mediated trans-formation. The PCR was used to detect the presence of the *nptII* gene among independent T_0 transgenic plants. Homozygous individuals were identified among T_1 seeds following kanamycin-resistant segregation of *nptII*.

Histochemical localization of β-glucuronidase activity: To localize the GUS activity, leaves, stems, petioles, fruits, and flowers of the transgenic plants were infiltrated with 1 mM 5-bromo-4-chloro-3-indolyl-glucuronide (X-Gluc) in 50 mM sodium phosphate buffer (pH 7) containing 0.5 mM $K_3Fe(SCN)_6$, 0.5 mM $K_4Fe(SCN)_6$, and 1 mM EDTA and incubated at 37 ºC overnight. After incubation, the plant material was cleared with 70 % (v/v) ethanol, and the leaves were photographed. Free-hand cross-sections were made from petioles and stems to visualize GUS activity at the cellular level. These cross-sections were rinsed in water and examined under transmitted white light. Fruits, leaves, and flowers were photographed under a dissecting microscope.

RNA extraction and cDNA generation: Leaf samples from tomato plants were frozen and homogenized in liquid nitrogen. RNA was extracted using the *EZ-RNA* kit (*Biological Industries*, Kibbutz Bet Haemek, Israel) with up to 0.5 cm^3 of frozen homogenized tissue per extraction tube. Two to three independent extractions were performed for each tissue set. The extractions were carried out according to the manufacturer's instructions. RNA pellets were suspended in 0.025 cm^3 of DEPC-treated H_2O and treated with DNase (*Ambion*, Austin, TX, USA) according to the manufacturer's instructions. The presence of RNA was confirmed by gel electrophoresis and DNA degradation was confirmed by PCR.

The RNA ($\leq$ 1 µg) from each sample was reverse-transcribed to cDNA using *MMLV RT* kit (*Promega*, Madison, WI, USA) in a 25-mm^3 reaction tube, with 2 mm^3 of random primers and 1 mm^3 of mixed poly-dT primers (18 to 23 nt) for the generation of cDNA from both rRNA and mRNA. All cDNA samples were diluted 1:8 with water.

Real-time expression analysis: Real-time reactions were prepared with *SYBR Green* mix (*Eurogentec*, Seraing, Belgium) in 10-mm^3 aliquots with 4 mm^3 of diluted cDNA per reaction, two replicates per cDNA sample. Reactions were run in a *RotorGene 6000* cycler (*Corbett*, Mortlake, Australia), 40 cycles per run, with sampling after each cycle. Results were interpreted with *RotorGene* software and normalized for each tissue set (three independent samples, two duplicates per sample). *Cyclophilin* was used as a reference gene for normalization.

Hydroponic cultivation: Seeds were planted on wet filter paper (Whatman®, *Schleicher & Schuell*, Dassel, Germany) in covered Petri dishes and left to germinate in the dark at room temperature. Three days after germination, the seedlings were moved to aerated hydroponic culture in 6.5-dm^3 containers filled with modified Hoagland solution (Hoagland and Arnon 1950, Damari-Weissler *et al.* 2009) and maintained in a growth chamber (an irradiance of 600 µmol m^{-2} s^{-1}, a 14-h photoperiod, day/night temperatures 25/21 °C, and an air humidity of 65 %). After one week, the young plants were transferred to 42-dm^3 boxes (12 plants per box) and the culture media were replaced twice a week.

Forced root exudation: The hydraulic conductance of tomato root systems was determined by measuring the flow induced in response to an applied pressure gradient (Gorska *et al.* 2008). De-topped root systems were fitted with a plastic tube filled with distilled water and connected to a beaker located on a balance ($\pm$ 0.01 mg). The root system was sealed in a chamber containing the hydroponic solution in which the plants had been grown. The pH of the solution was kept at ~6.1 using 2-(*N*-morpholino) ethane sulfonic acid (MES) buffer (1 g dm^{-3}). The pressure in the chamber was regulated using a needle valve, which was adjusted to allow a small leak in the chamber, such that air used to pressurize the chamber also served to aerate the medium. Water flow through the root system was automatically recorded by a computer at 30 s intervals. Flow stabilization occurred

10 to 20 min after the plant was exposed to pressure. Flow data were then collected for 1 h. At the end of each experiment, the fresh mass of the roots was recorded. The roots were then dried in an oven at 90 °C for 72 h, after which the dry mass was recorded and then root flow was calculated according to Gorska *et al.* (2008).

Stem hydraulic conductivity: Small sections of stems (~5 cm long) were cut under water to prevent embolisms caused by air entering into the cut vessel. In addition, stems were perfused under elevated pressure (150 kPa) for few minutes to remove pre-existing embolism. The hydraulic conductivity (K) of each stem segment was measured gravimetrically by determination of flow rate of filtered and degassed 10 mM KCl solution. A solution was located on a balance (*Sartorius*; precision $\pm$ 0.1 mg) and connected to the stem by a plastic tube. The stem was submerged in a water bath with the water level being ~10 cm below the level of water on the balance. After a steady flow rate was reached (within just a few minutes), the specific hydraulic conductivity (Kh) was calculated as volumetric flow rate [kg s^{-1}] divided by the pressure difference across the stem length [MPa m^{-1}]. Kh was then normalized either by xylem cross-sectional area [m^2] or leaf area [m^2] to calculate either Ks (xylem specific conductivity) or Kl (leaf specific conductivity) [kg s^{-1} m^{-1} MPa^{-1}]. Xylem cross-sectional area was microscopically determined for each stem. Leaf area was determined for all leaves above the cut section using digitized leaves images.

Anatomical techniques: To analyze the anatomy of all of the plants, stem samples were taken from at least two independent plants from each line at the same time and stored in 70 % (v/v) ethanol until examination. Free-hand cross-sections were taken, stained for a few seconds in 2 % (m/v) lacmoid (*Sigma-Aldrich*, St. Louis, MO, USA) in 96 % (v/v) ethanol and then rinsed in tap water for a few minutes, mounted in 50 % (m/v) sodium lactate (Aloni 1980), and observed under transmitted white light. The lacmoid stained (marine blue) the lignin in the cell walls of the vessels and the fibers. Cross-sections were also stained with phloroglucinol and mounted in phloroglucinol-HCl for specific lignin staining (Ruzin 1999).

Statistical analysis was performed using the *JMP 5.0* software program. Means were compared using Student's *t*-test.

Results and discussion

To determine in which tissues *SlFRK1* is expressed, we isolated a 1.7 Kb fragment of the *SlFRK3* promoter region including the 5' UTR, fused it to the β-glucuronidase (*GUS*) reporter gene and used it to generate transgenic tomato plants. Three independent transgenic lines showed GUS staining in the vascular tissues of their

leaves. Thorough promoter expression analysis revealed that the *SlFRK1* promoter was expressed in stamens and in vascular tissues of leaves, petioles, stem, roots, and fruits, (Fig. 1). The *SlFRK1* promoter, similar to the *SlFRK2* and *SlFRK3* promoters, was expressed primarily in the stem secondary xylem (Fig. 1*E*). Yet, while the

SlFRK2 promoter is expressed throughout the entire secondary xylem (Fig. 1*E* Suppl.) and the *SlFRK3* promoter was expressed mainly in the cambium and newly differentiating xylem cells (Fig. 2*C* Suppl.), the *SlFRK1* promoter was expressed primarily in the mature secondary xylem and not in the cambium or differentiating xylem (Fig. 1*E*). In addition, the *SlFRK1* promoter was expressed in phloem companion cells of both internal and external phloem (Fig. 1*C,F*). This was different from *SlFRK2* and *SlFRK3*, whose promoters were expressed in either internal or external phloem, respectively (Figs. 1*F*, 2 Suppl.).
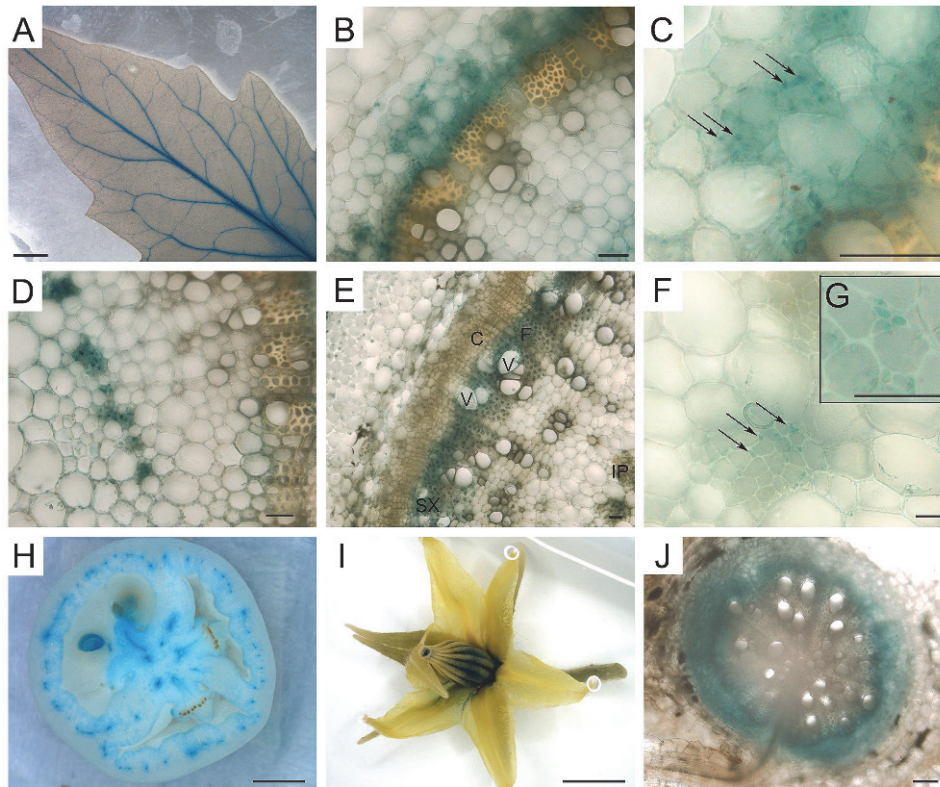

Fig. 1. Histochemical analysis of the expression pattern of the *SlFRK1* promoter visualized by GUS staining in tomato tissues:. *A* - leaf, *B,C* - cross-sections of leaf petiole, *D-G* - stem cross-sections, *H* - young green fruit, *I* - opened flower, and *J* - primary root (C - cambium, F - xylem fibers, V - vessel, SX - secondary xylem, IP - internal phloem; *black arrows* - phloem companion cells; *scale bars*: *A,H,I* - 5 mm, *B-G,J* - 50 µm).
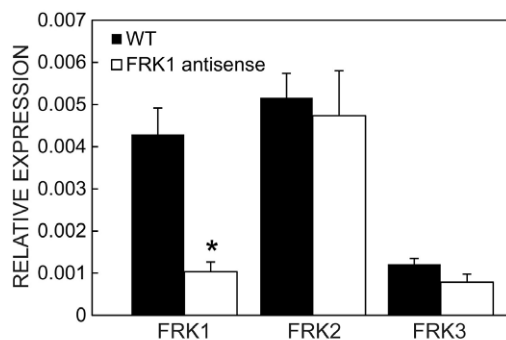

Fig. 2. Expression of *SlFRK* genes in leaves of the FRK1-antisense line. Samples were taken from leaf 4. Relative expression of three *SlFRK* genes in the FRK1-antisense line and WT plants was determined by real-time expression analysis with gene-specific primers (German 2003). Expression was normalized to the expression of cyclophylin in each sample. Means ± SEs, *n* = 3, * - a statistically significant difference (*t*-test; *P* < 0.05).

The FRK-promoter expression patterns in the vascular tissues (Fig. 1) correlate well with fructose-phosphorylation activity, which was found to be 2- to 3-times higher in the secondary xylem than in the pith or bark (German *et al.* 2003). The complex expression patterns of tomato FRKs together with the specific roles of FRK2 in the development of tracheary elements (Damari-Weissler *et al.* 2009) and FRK3 and FRK2 in xylem fiber development (Stein *et al.* 2016) suggest that FRK1 may also play a specific and unique role in vascular development.

Fructose phosphorylation may be crucial for fructose metabolism in various non-photosynthetic cells and we might expect to detect expression of at least one FRK isozyme in other sink cells, such as the cells of the root cortex. One possible reason for the lack of *GUS* expression in other types of cells is that HXKs are sufficient for fructose phosphorylation, despite their relatively low affinity for fructose. But, it is also possible that the 1.5 - 2 kb promoter fragments used for the tomato

FRK gene expression analyses might not include all the regulatory elements responsible for gene expression. A good example of that type of scenario is the tomato sucrose transporter 1 (*LeSUT1*), in which some of the regulatory sequences promoting expression in phloem companion cells, trichomes, and guard cells are found in introns (Weise *et al.* 2008). Nevertheless, the analyses of GUS expression provide a strong indication of the putative roles of FRKs in vascular tissues.

To study the role of *SlFRK1 in planta*, we generated transgenic tomato plants with an antisense fragment of *SlFRK1* expressed under the 35S promoter. Seven independent lines were created and confirmed as transgenic by PCR. Homozygous plants were identified among T_1 plants using kanamycin-resistance segregation analysis. Out of the seven homozygous lines, only one line, a84, showed reduced expression of the *SlFRK1* gene (Fig. 3 Suppl.). The a84 transgenic line, which will be referred to from now on as the FRK1-antisense line, showed specific FRK1 suppression, with about 77 % reduction in *SlFRK1* expression, but no effects on the expression of *SlFRK2* or *SlFRK3* (Fig. 2).

Under normal growing conditions, the FRK1-antisense plants did not show any altered phenotype, relative to WT plants. However, FRK1-antisense plants had reduced water conductance in their stems (Fig. 3*A,B*), but not in their roots (Fig. 3*C*). This effect is different from that observed in plants with specific suppression of either FRK2 or FRK3, in which both stem and root hydraulic conductivity were reduced (Damari-Weissler *et al.* 2009, Stein *et al.* 2016). Although all three promoters were found to be expressed in the vascular tissue of roots, real-time PCR results showed that *SlFRK1* expression in roots is very low compared to that of *SlFRK2* and *SlFRK3* (German *et al.* 2004). This may be the reason why FRK1 suppression had no effect on root hydraulic conductivity. These results may indicate that FRK1 plays a primary role in stems. Yet, unlike suppression of FRK2, which has significant anatomical effects on the stem vascular tissues (Damari-Weissler *et al.* 2009), no clear anatomical effects were observed in FRK1-antisense stems (Fig. 5*B*).

In a previous study, we demonstrated that specific suppression of FRK3 also reduced stem hydraulic conductivity with no effects on vascular anatomy, but combined suppression of FRK2 and FRK3 had very significant anatomical effects, indicating that FRK2 and FRK3 may compensate for one another in vascular development. To explore compensation between FRK1 and FRK2 or FRK3, we crossed FRK1-antisense plants with either FRK2-antisense or FRK3-RNAi plants that had specific suppression of FRK2 or FRK3, respectively.

Homozygous F_2 plants of a cross between a FRK1-antisense line and a FRK3-RNAi (F3R1) line did not show altered growth and the plants appeared similar to WT plants (Fig. 4), suggesting that the two genes might not be expressed in the same cells or that *FRK2* might be able to compensate for the decreases in both *FRK1* and *FRK3*. However, plants homozygous for antisense suppression of both FRK1 and FRK2 showed severe growth inhibition even relative to FRK2-antisense plants with more pronounced effects on leaf wilting than those observed among FRK2-antisense plants (Fig. 4). The additive effect of FRK1 and FRK2 suppression was quite similar to the additive effect observed in plants with combined suppression of FRK2 and FRK3 (Fig. 4), which also resulted in very stunted plants that were unable to grow taller than 20 cm and did not flower and set fruit. These results suggest that FRK1 contributes to tomato vascular development together with FRK2.
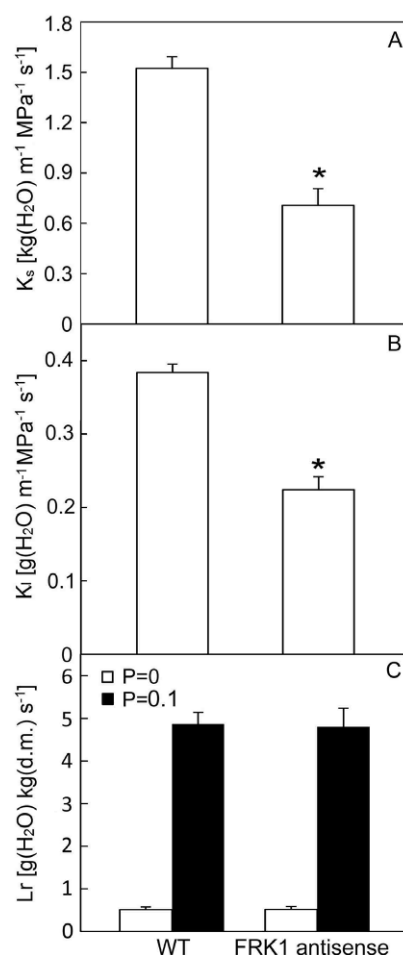

Fig. 3. The effect of *SlFRK1* suppression on stem and root hydraulic conductivity. *A* - stem hydraulic conductivity (Ks) normalized to xylem cross-sectional area; *B* - stem hydraulic conductivity (Kl) normalized to leaf area. Means ± SEs, *n* = 8, * - statistically significant differences (*P* < 0.05) between WT and FRK1-antisense plants. *C* - Root conductivity (Lr) was determined based on pressure-driven exudation (*black bars*, pressure = 0.1 MPa) and osmotically generated flow (*open bars*). Means ± SEs, *n* = 4.

To better understand the impact of FRK1 on tomato vascular development, we analyzed the anatomy of the homozygote double-mutant FRK1-antisense × FRK2-antisense plants. Cross-sections taken from stems showed that the width of the secondary xylem was drastically

reduced compared to WT stems and to the stems of each of the independent parent lines (Fig. 5), indicating reduced cambial activity. In addition, the double-mutants secondary xylem contains much fewer vessels (Fig. 5*D*). However, since these plants are very stunted, it might be expected that they would have less secondary xylem, making it impossible to determine whether the reduced secondary xylem is the cause or the outcome of the growth inhibition.
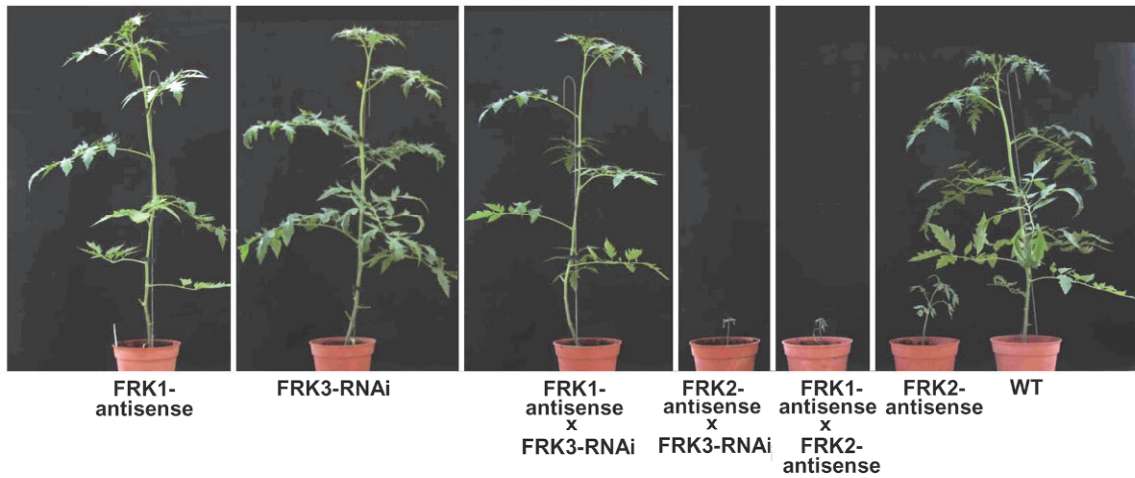

Fig. 4. Co-suppression of *SlFRK1* and *SlFRK2* enhanced growth inhibition. Plants were grown under normal conditions in the greenhouse and photographed about 10 weeks after germination.
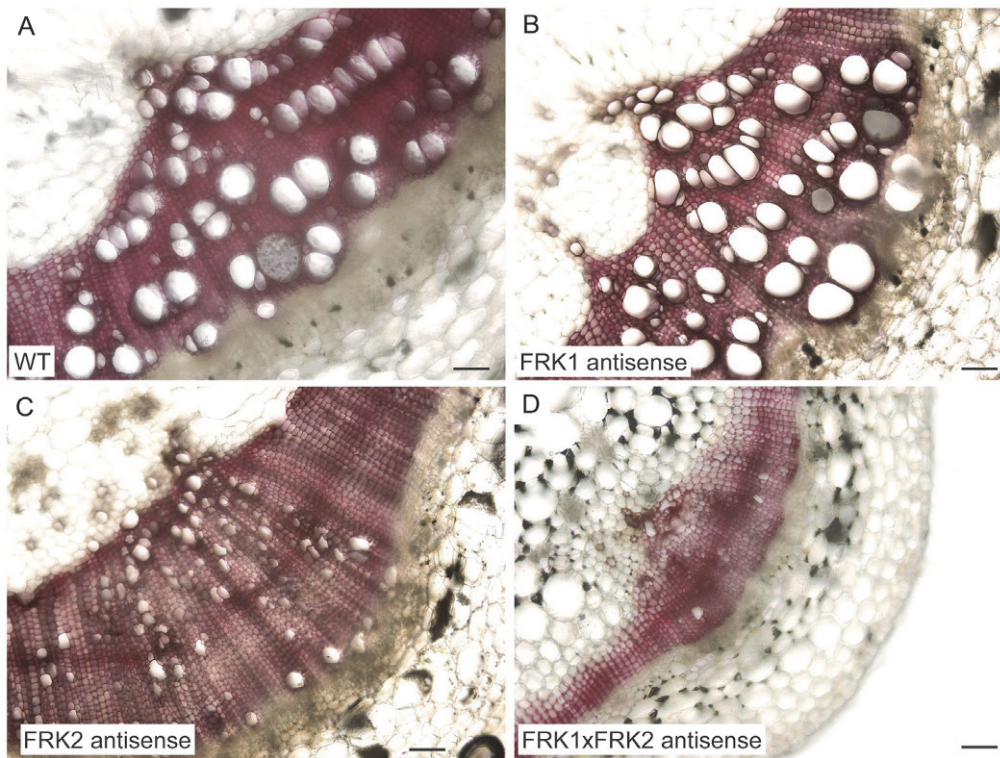

Fig. 5. Co-suppression of *SlFRK1* and *SlFRK2* reduced secondary xylem area. Stem cross-sections of 10-week-old plants shown in Fig. 4 taken from the internode between the first and second true leaves, showing reduced secondary xylem width (*stained pink*) and reduced vessel width. *Scale bars* - 100 μm.

Interestingly, phloroglucinol stained lignin in the xylem fibers, but did not in the phloem fibers in all the lines (Fig. 5), suggesting that phloem fibers are less sensitive to phloroglucinol stain, possibly due to less lignin or different lignin properties. Similar differential staining of xylem and phloem fibers has been observed in tobacco stem cross-sections (Townsley *et al.* 2013). Although phloroglucinol did not stain the lignin in the

phloem fibers, cross-sections of the F2 homozygous FRK1 and FRK2 antisense plants clearly revealed that some phloem fibers had narrower cell walls and some were distorted (Fig. 4 Suppl.) suggesting *SlFRK1* might be important for phloem fiber development. Distorted and thin cell walls of phloem fibers were previously observed

in stems of plants of the FRK3-RNAi line (Fig. 5 Suppl.), F3R4, which had decreased expression of all three FRKs (Stein *et al.* 2016). However, it is not clear which of the FRKs contributed to the distorted phloem fibers in this phenotype.
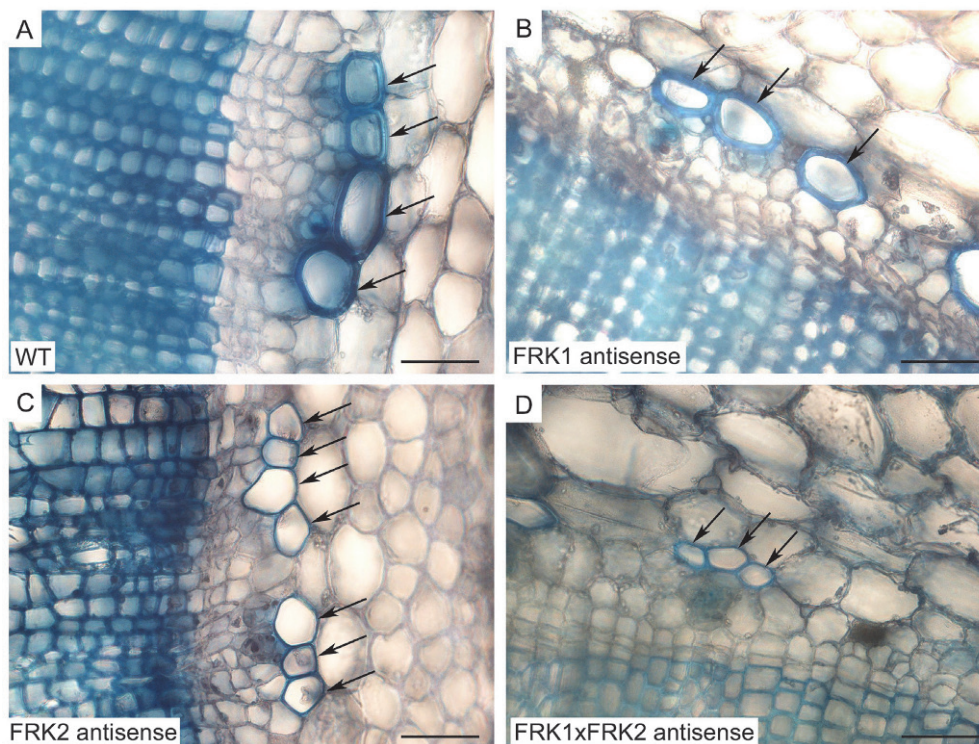

Fig. 6. Co-suppression of *SlFRK1* and *SlFRK2* reduced the size of phloem fiber cells. Stem cross-sections of 10-week-old plants shown in Fig. 4 taken from the internode between the first and second true leaves, showing smaller phloem fibers (*arrows*). *Scale bars* - 50 μm.

To better elucidate the effect on phloem fibers, we analyzed the stems using lacmoid staining that stain phloem fibers. Inspection of the phloem fibers in the FRK1-antisense × FRK2-antisense plants (Fig. 4) revealed that those plants had smaller phloem fiber cells than WT, FRK1-antisense, or FRK2-antisense plants (Fig. 6). These phloem fibers were not only smaller, but also had narrower cell walls with less lignin and, therefore, were not always stained by the lacmoid (Fig. 7*D*), in contrast to the phloem fibers in FRK2-antisense × FRK3-RNAi plants, which were stained very well with lacmoid, to reveal thick and normal cell walls (Fig. 7*C*). Some variability in the effects on phloem fibers of FRK1-antisense × FRK2-antisense plant was observed, with a few phloem fibers stained with lacmoid and stained fibers usually possessing thin cell walls (Fig. 7*E,F*). We assume that this variability is a result of the differential suppression of *SlFRK1* or *SlFRK2*. Nevertheless, the appearance of this phenotype in the FRK1-antisense × FRK2-antisense double-mutant and not in FRK2-antisense × FRK3-RNAi plants or in FRK1-antisense × FRK3-RNAi plants indicates that it is caused

by the specific co-suppression of *SlFRK1* and *SlFRK2* and implies a specific role for FRK1 in phloem fiber development together with FRK2.

The decrease in phloem fiber cell-wall width and lignification together with no obvious effects on the surrounding cells implies reduced metabolism and the reduced ability of these cells to utilize the sugars for the synthesis of cell-wall components such as cellulose or lignin. These findings are congruent with those of a previous study that found that the FRK in aspen wood is very important for cell-wall metabolism through carbon partitioning to cellulose in xylem fibers (Roach *et al.* 2012).

Interestingly, unlike FRK2, FRK1 is not inhibited by fructose and its affinity for fructose is much lower than that of FRK2. It is not clear why some FRKs are inhibited by fructose while others are not. We speculate that FRK1 may complement FRK2, whose activity is inhibited by fructose, perhaps when the concentration of fructose rises in certain cells that need high levels of carbon metabolism, such as developing trachea vessels and phloem fibers that have thick cell walls.
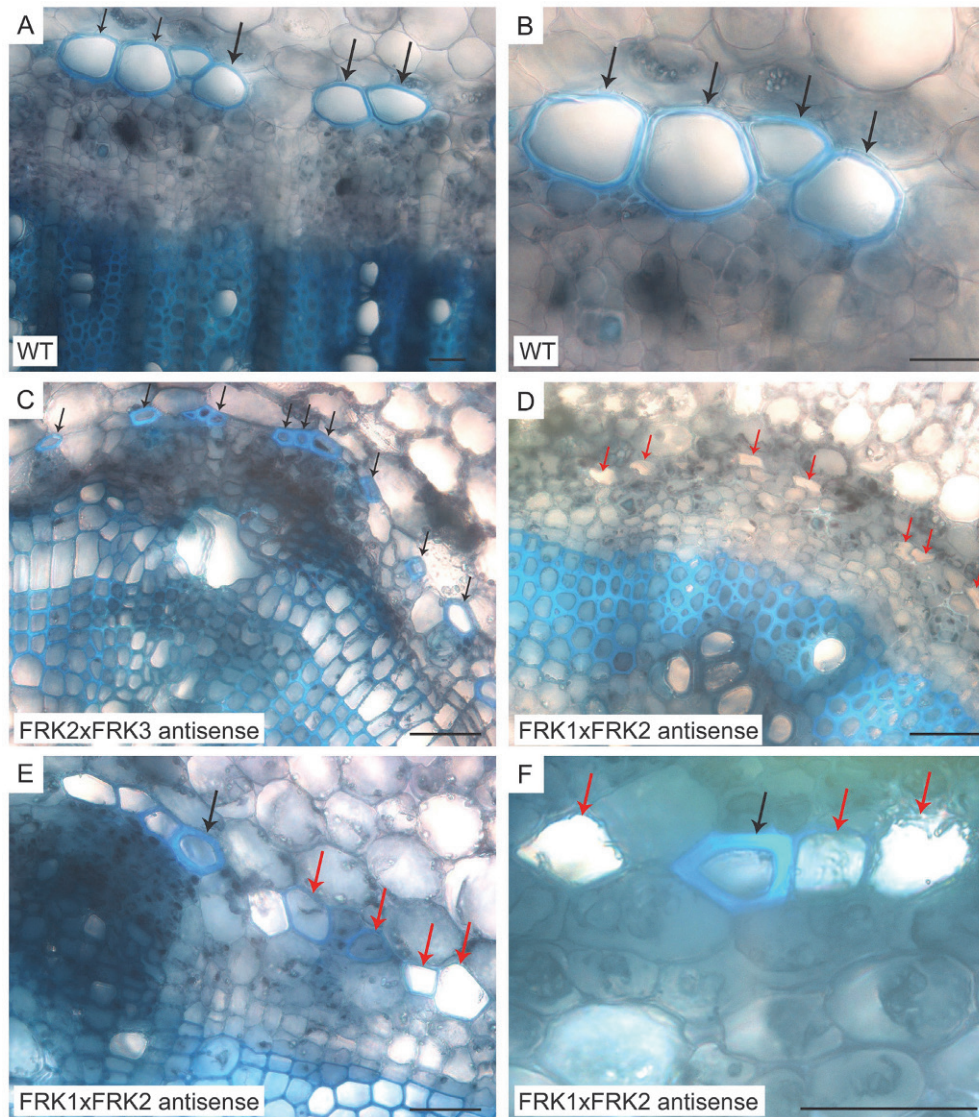
Fig. 7. Co-suppression of *SlFRK1* and *SlFRK2* reduced the lignification and cell-wall width of phloem fiber cells. Stem cross-sections of 10-week-old plants taken from the internode between the first and second true leaves. *Black arrows* - normal phloem fibers; *red arrows* - thin cell-wall with reduced lignification in phloem fibers. *Scale bars* - 50 µm.

Conclusions

This work illustrates the importance of the cytosolic FRK1 for tomato vascular development and adds to our understanding of the roles of the other two major FRKs, the cytosolic FRK2 and the plastidic FRK3, in vascular development. The FRK1 was found to be important for water conductance through vessels and for phloem fiber development alongside FRK2. The main effects observed after *SlFRK1* suppression were reduced cell-wall width and reduced lignification resulting in distorted xylem vessels and phloem fibers, indicating that fructose phosphorylation in the vascular tissues is highly important for cell-wall metabolism.

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