Final Scientific Report

Cover Page

BARD Project Number: IS-3897-06

Date of Submission of the report: January 6th 2011

Project Title: Role of Fructokinases in the Development and Function of the Vascular System

Investigators

Principal Investigator (PI): David Granot

Co-Principal Investigator (Co-PI): Noel M Holbrook

Collaborating Investigators:

Institutions

The Volcani Center

Harvard University

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.
Fructokinase, Fructose phosphorylation, Vascular tissues, Xylem, Phloem

Abbreviations commonly used in the report, in alphabetical order:
FRK - fructokinase; GUS - beta glucuronidase

Budget: IS: \$ 285,000

US: \$ 171,000

Total: \$ 114,000

David Granot

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1		4	
Submitted, in review, in preparation	2			
Invited review papers				
Book chapters			1	
Books				
Master theses				
Ph.D. theses			1	
Abstracts			7	
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	2	3	6
Longer Visits (Sabbaticals)				

Description Cooperation:

Our collaboration is highly complementary in terms of our skills and perspective and was very fruitful. The transgenic tomato lines created in Granot's lab were analyzed in Holbrook's lab for hydraulic properties and many other physiological characteristics.

The PI's met already six times; in a conference of ASPB focus meeting on The Biology of Transpiration, Snowbird, Utah (October 2006), in the American Society of Plant Biology (ASPB) Joint Congress, Chicago (July 2007), and in Plant Vascular Biology meeting in Columbus Ohio (July 2010). David Granot spent twice a week in Holbrook's lab, on February 2008, and on January 2009, and Noel Holbrook visited Granot's lab on April 2009. In additions, Shimon Rachmilevitch, a post doc at Holbrook's lab working on this subject, visited the laboratory of Granot. A joint MS has been published and two more are being prepared.

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A continuation proposal has been submitted.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

Plant vascular tissues are superhighways whose development and function have profound implications for productivity, yield and stress response. Preliminary studies by the PI indicated that sugar metabolism mediated by fructokinases (FRKs) has a pronounced effect on the transport properties of the xylem. The goal of this research was to determine how the main fructokinase gene, *FRK2*, and the only plastidic fructokinase, *FRK3*, influence vascular development and physiology, emphasizing processes that occur at both the cellular and organismic level. We found that both genes are expressed in vascular tissues, but *FRK3* is expressed primarily in vascular tissues of mature petioles. Vascular anatomy of plants with antisense suppression of *FRK2* uncovered that *FRK2* is necessary for xylem and phloem development, most likely due to its role in vascular cell-wall synthesis, and affects vascular development all over the plant. As a result, suppression of *FRK2* reduced hydraulic conductivity of roots, stem and leaves and restricted sugar phloem transport. Vascular anatomy of plants with RNAi suppression of *FRK3* uncovered that *FRK3* is required for vascular development in mature petiole but its role is partially complemented by *FRK2*. Suppression of *FRK3* combined with partial suppression of *FRK2* had effects completely different from that of *FRK2* suppression, resulting in wilting of mature leaves rather than young leaves of *FRK2* suppressed plants, and decreased export of photoassimilates. This primary effect of *FRK2* suppression on mature petioles had a secondary effect, reducing the hydraulic conductivity in roots and stem. The very fact that a plastidic fructokinase plays a role in vascular development is quite surprising and we are still seeking to uncover its metabolic mode-of-action. Yet, it is clear that these two fructokinases have different roles in the coordination between photosynthetic capacity and vascular development. We have started analyzing the role of the last third FRK, *FRK1*, and discovered that it is also expressed exclusively in vascular tissues. It appears therefore, that all FRKs studied here are involved in vascular development.

An interesting unexpected outcome of this study was the connection of *FRK2* with hormonal regulation of vascular development, most likely auxin. This observation together with the yet to be solved questions on the exact roles of *FRK3* are the subjects of our current efforts.

Research Objectives

1. Tissue localization of *FRK2* and *FRK3* expression
2. Detailed vascular anatomy of roots, stems, and leaves of *FRK2*- and *FRK3*-antisense plants

3. Analyze the effect of *FRK2*- and *FRK3* suppression on the hydraulic conductivity and vulnerability to cavitation of roots, stem, petioles and leaves
4. Assess the effect of *FRK2*- and *FRK3*- suppression on phloem transport
5. Explore how *FRK2*- and *FRK3*- suppression influence the coordination between photosynthetic capacity and xylem development
6. Assay the effect of *FRK2* and *FRK3* suppression on vascular gene expression

Achievements as related to the original research proposal and objectives

1. Tissue localization of *FRK2* and *FRK3* expression

Transgenic tomato plants expressing *FRK1_{promoter}::GUS*, *FRK2_{promoter}::GUS* and *FRK3_{promoter}::GUS* were generated. *FRK1_{promoter}::GUS* is expressed in vascular tissues of leaves and petiole (Fig. 1A&C), and in stem inner and outer phloem (Fig. 1B). Initial analysis of *FRK2_{promoter}::GUS* expressing plants indicates expression in vascular tissues (Fig. 1E&F). *FRK3_{promoter}::GUS* is expressed specifically in the cambium region of mature petioles (Fig. 1D), in line with the phenotype of *FRK3* repressed plants (see below). It is worth noting that the three *FRKs* have different expression pattern in petioles (Fig. 1C,D&F).

2. Detailed vascular anatomy of roots, stems, and leaves of *FRK2*- and *FRK3*-antisense plants

Detailed vascular anatomy of *FRK2*-antisense plants has been done and has already been published (Damari-Weissler et al., 2009). Suppression of *FRK2* inhibited development of all cell types in the vascular tissues including cambium, xylem and phloem (Damari-Weissler et al., 2009). The secondary xylem is narrower, xylem vessels are smaller, xylem cell-wall is thinner, and the cambium has less cell-layers. These observations suggest that *FRK2*, the major fructokinase expressed in tomato plants, is necessary for proper development of the vascular system, and its suppression probably reduces sucrose cleavage and cellulose synthesis (Damari-Weissler et al., 2009).

Figure 1: Expression (blue color) of *FRK1_{promoter}::GUS* (A, B, C), *FRK3_{promoter}::GUS* (D) and *FRK2_{promoter}::GUS* (E, F) in vascular tissues of tomato plants. IP-inner phloem; OP-outer phloem

Vascular anatomy of *FRK3*

repressed plants revealed that only mature petioles developed aberrant vascular tissues with necrotic regions (Fig. 2B). Cross sections of the stem/petiole junction

demonstrate that the effect is

restricted to the petiole (Fig. 2D).

These observations suggest that

FRK2, the major fructokinase

expressed in tomato plants, is

necessary for proper development of

the vascular system, and its suppression probably reduces sucrose cleavage and cellulose synthesis.

Figure 2: Cross sections in petioles (A&B) and in leaf-stem junction (C&D) of wild type and F3R4 plants. The red circle mark abnormal brownish disrupted vascular tissues.

3. Analyze the effect of *FRK2*- and *FRK3* suppression on the hydraulic conductivity and vulnerability to cavitation of roots, stem, petioles and leaves

We found that water conductance of *FRK2* antisense plants is reduced in stems, roots and leaves, suggesting that *FRK2* influences xylem development along the entire vascular system. Interestingly, the build-up of positive xylem pressure under static (no-flow) conditions was decreased. These results were published in (Damari-Weissler et al., 2009).

Transgenic tomato plants with specific suppression of the plastidic *FRK3* (Fig. 3, F3R1 line) had no visible phenotypic effects, but did have 35% lower hydraulic conductivity in

stem and roots. Interestingly, suppression of *FRK3* accompanied with partial suppression of *FRK2* (line F3R4) had clear visible phenotypic effects, different from that of *FRK2*-antisense plants (Fig. 3). While *FRK2*-antisense plants had wilted young leaves (Damari-Weissler et al., 2009), the 3R4 plants exhibited wilting of mature leaves (Fig. 3), and had 75% reduced stem hydraulic conductivity and 25%

Figure 3: Effects of *FRK3* and *FRK2* suppression. A. Relative expression of the three FRK genes in different *FRK3*-RNAi transgenic lines. B. Phenotypic effects of *FRKs* suppression. The yellow arrows point to wilting mature leaves.

reduced root conductivity. However, double and triple grafting experiments clearly demonstrated that the wilting and the growth inhibition were imposed entirely by the leaves (Fig. 4). These observations are in line with the cross section results described above showing that only mature petioles developed aberrant vascular tissues with necrotic regions (Fig. 2).

4. Assess the effect of *FRK2*- and *FRK3*-suppression on phloem transport

To examine the effect of *FRK2* suppression on phloem transport, we created triple grafted plants in which an antisense interstock replaced a wild type stem portion, and analyzed the sugar content in the different portions of the triple grafts. Sucrose content above and in the *FRK2*-antisense interstock was significantly higher than below the graft. These results show that the antisense interstock restricted downward sugar movement, suggesting that *FRK2* is required for both phloem and xylem development. Yet, triple graft experiments demonstrate that restricted upward water transport through the xylem and not interruption of downward sugar transport in the phloem is the underlying cause of the growth inhibition and wilting phenotype of *FRK2*-antisense plants (Damari-Weissler et al., 2009).

The major effect on phloem transport in *FRK3* suppressed plants appears to be on sugar export of sugars from mature leaves. As a result, sugar content in stem is lower compare to that of control plants, which is probably the primary cause for the severely reduced stem thickness and for the abortion of most of the flowers.

5. Explore how *FRK2*- and *FRK3*- suppression influence the coordination between photosynthetic capacity and xylem development

As described above, to assess the effect of *FRK2*- suppression on phloem transport we created triple grafted plants containing an antisense-*FRK2* stem portion as an interstock. Overtime, this interstock becomes thick (Fig. 5). Cross section of the thickening portion uncovered a spectacular phenomenon of ring formation, of alternating xylem vessels and fibers (Figure 6). These rings could stem from hormones (auxin and gibberellin) pulses, in

Figure 4: Reciprocal (A) and triple (B) grafts. The roots and stem of F3R4 are insufficient to impose growth inhibition and wilting, indicating a role for the leaves. Red circles – wilting mature leaves.

which auxin from young leaves stimulate formation of xylem vessels while gibberellins from mature leaves stimulate formation of fibers. We believe that this interesting observation about the potential connection between *FRK2* and hormonal regulation of vascular tissues reflects on the coordination between photosynthetic capacity and xylem development.

Figure 5: Thickening of *FRK2*-antisense interstock. w.t.-wild type

Figure 6: Cross sections of wild type interstock (A) and *FRK2*-antisense thick interstock (B) and C (enlarged). The rings are composed from alternating lignified fibers (red arrows) and unlignified trachea (xylem vessels - yellow arrows).

6. Assay the effect of *FRK2* and *FRK3* suppression on vascular gene expression

This part of the work has not yet been done, as we were very much occupied by the other parts of the research that proved to be very fruitful.

Description of the cooperation

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A continuation proposal has been submitted.

List of publications

The following two publications stemmed from the work on this project:

1. Damari-Weissler, H., Ginzburg, A., Gidoni, D., Mett, A., Krassovskaya, I., Weber, A.P., Belausov, E., and Granot, D. (2007). Spinach *SoHKK1* is a mitochondria-associated hexokinase. *Planta* 226, 1053-1058.
2. Granot, D. (2007). Role of tomato hexose kinases. *Functional Plant Biology* 34, 564-570.
3. Granot D. (2008). Sugar metabolism and signaling in plants. In " A Transgenic Approach to Plant Biochemistry and Physiology" Editors: Marisela Rivera-Domínguez, Rosalba-Troncoso Rojas and Martín Ernesto Tiznado-Hernández pp 51-72 Kerala, India
4. Granot D. (2008). Putting plant hexokinases in their proper place. *Phytochemistry* 69: 2649-2654
5. Damari-Weissler H., Rachmilevitch S. Aloni R., German M.A., Cohen S., Zwieniecki M.A., Holbrook N.M. and Granot D. (2009). *LeFRK2* is required for phloem and xylem differentiation and the transport of both sugar and water. *Planta* 230: 795-805
6. Swartzberg D., Hanael R. and Granot D. (2010). Relationship between hexokinase and cytokinin in the regulation of leaf senescence and seed germination. *Plant Biology*, DOI: 10.1111/j.1438-8677.2010.00376.x