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BARD

FINAL REPORT

PROJECT NO. US-3231-01

**Biotechnology Approaches to Enhance Phosphorus
Acquisition of Tomato Plants**

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2006

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Cover Page

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Project Title: Biotechnology approaches to enhance phosphorus acquisition of tomato plants

Investigators

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**Co-Principal Investigator (Co-PI):
AVNER SILBER**

**Collaborating Investigators:
AVRAHAM LEVY**

Institutions

PURDUE UNIVERSITY

VOLCANI CENTER

WEIZMANN INSTITUTE

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Phosphate transporter, phosphatase, transformation, phosphate nutrition
Abbreviations commonly used in the report, in alphabetical order:

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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

Ajay Jain **Purdue University**
Karthikeyan A.S. **Purdue University (Partial support)**
Maria Vasconcelos **Purdue University (partial support)**
Gua-hua Xu **Volcani Center**

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	one	Two		Three
Longer Visits (Sabbaticals)				

Description Cooperation:

This project has resulted in one of the productive collaboration between the scientists from USA and Israel. The groups of scientists from both countries worked together on several research projects. This includes the characterization of a novel phosphate transporter and a phosphatase. Recent results of these scientific interactions are being prepared for two publications. This interaction has led to sharing of biological and molecular materials and tools between the laboratories. This has also helped in training several postdoctoral research associates and a graduate student. The genetic resources and molecular tools developed during this research are being used by two Ph.D. students at Purdue University. The investigators are in constant communication and exploring further opportunities for continued collaborations.

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Visit by collaborators. During the year 2002 one of the collaborators Dr. Avi Levy from Israel visited the laboratory of principal investigator at Purdue University. This visit helped in consolidating research plans and exchange of ideas and research materials. Dr. Avi Levy provided the LePT3 (phosphate transporter) mutant seeds for molecular analysis at Purdue. He also presented a seminar on research activities in his laboratory. Dr. Avner Silber visited Purdue during this grant period and held useful discussion on research and future collaborative efforts between the laboratories. In 2005 Dr. Raghothama visited Israel and delivered two seminars at Volcani center, Bet Dagan and at the Weizmann Institute of Science. This visit was very fruitful in reviewing the project activities and planning for future collaborations. This has helped us to establish a durable and productive relationship among the partners. Dr. Avner Silber will be visiting Purdue University in April 2006 to deliver a seminar.

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Abstract: Phosphorus is one of the least available macronutrient in the soil. The high affinity phosphate transporters are known to be associated with phosphate acquisition under natural conditions. Due to unique interactions of phosphate with soil particles, up to 80% of the applied phosphates may be fixed forcing the farmers to apply 4 to 5 times the fertilizers necessary for crop production. Efficient uptake and utilization of this essential nutrient is essential for sustainability and profitability of agriculture. Many predictions point to utilization/exhaustion of high quality phosphate rocks within this century. This calls for efforts to improve the ability of plants to acquire and utilize limiting sources of phosphate in the rhizosphere. Two important molecular and biochemical components associated with phosphate efficiency are phosphate transporters and phosphatases. This research project is aimed at defining molecular determinants of phosphate acquisition and utilization in addition to generating phosphate uptake efficient plants. The main objectives of the project were;

- 1) Creation and analysis of transgenic tomato plants over-expressing phosphatases and transporters
- 2) Characterization of the recently identified members (LePT3 and LePT4) of the Pi transporter family
- 3) Generate molecular tools to study genetic responses of plants to Pi deficiency

During the project period we have successfully identified and characterized a novel phosphate transporter associated with mycorrhizal symbiosis. The expression of this transporter increases with mycorrhizal symbiosis. A thorough characterization of mutant tomato lacking the expression of this gene revealed the biological significance of LePT3 and another novel gene LePT4. In addition we have isolated and characterized several phosphate starvation induced genes from tomato using a combination of differential and subtractive mRNA hybridization techniques. One of the genes, LePS2 belongs to the family of phospho-protein phosphatase. The functionality of the recombinant protein was determined using synthetic phosphor-peptides. Over expression of this gene in tomato resulted in significant changes in growth, delay in flowering and senescence. It is anticipated that phospho-protein phosphatase may have regulatory role in phosphate deficiency responses of plants. In addition a novel phosphate starvation induced glycerol 3-phosphate permease gene family was also characterized. Two doctoral research students are continuing the characterization and functional analysis of these genes. Over expression of high affinity phosphate transporters in tobacco showed increased phosphate content under hydroponic conditions. There is growing evidence suggesting that high affinity phosphate transporters are crucial for phosphate acquisition even under phosphate sufficiency conditions. This project has helped train several postdoctoral fellows and graduate students. Further analysis of transgenic plants expressing phosphatases and transporters will not only reveal the biological function of the targeted genes but also result in phosphate uptake and utilization efficient plants.

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Achievements

The goals of this project are to produce tomato plants efficient in acquiring Pi from naturally limiting condition, and to learn the genetic and molecular regulation of Pi acquisition in plants. We took multiple approaches to accomplish the stated objectives. We have screened the M3-seeds of several mutants of fast-neutron irradiated M82-M2 and EMS treated Micro-Tom plants which were tolerant to low-P (0.025 mM) or resistant to high-P (6 mM) supply. This screening yielded mutants containing higher leaf P under low P condition. Further analysis of mutants resulted in the isolation of a new member of the high affinity phosphate transporter *LePT4*.

In the absence of mycorrhizae infection, *LePT4* expression was very low in the wild type and a mutation in this gene only slightly reduced total P uptake in tomato plants grown under high Pi (1mM). Under Pi starvation (0.05mM), the mutant had severe P deficiency symptoms and uptake was lower than in the wild type. At very high P (5mM) the *lept4* mutant was more resistant than the wild type whose growth was inhibited. These data suggest that *LePT4* contributes to P uptake under low Pi independently of mycorrhizae. Nevertheless, the role of *LePT4* was strongly emphasized upon mycorrhizal infection and low Pi. This suggests that *LePT4* is a major but not unique route for Pi acquisition via mycorrhizae in tomato. We have identified another gene, *LePT3* a tomato gene orthologous to *StPT3* that is also enhanced upon mycorrhizal infection. Two manuscripts highlighting efforts of US and Israeli partners are in the final stages of preparation and resubmission for publication.

Molecular analysis of the promoter of high affinity Pi transporter (*AtPT2*) of Arabidopsis showed Pi starvation mediated expression of reporter genes. This promoter was characterized for the temporal, spatial and Pi regulated expression, and appears to be ideal for expressing genes in tomato. Several lines of tomato plants over-expressing the Pi starvation induced phosphatase gene (*LePS2*) had distinct phenotypic traits and they exhibited phosphate deficiency symptoms. *LePS2* was determined to be a bona-fide phosphoprotein phosphatase. Over expression of this gene reduced plant height, delayed flowering and resulted in anthocyanin accumulation during the early periods of growth. However the growth rate recovered in the over-expression lines thus expanding life span of the plants. The salient feature of these transgenic plants is the extended plant life leading more branches and more flowers. We have also identified several other homologs of *LePS2* in tomato and Arabidopsis. Studies are underway to define the biological function of *LePS2* family of genes. In addition our work also has resulted in isolation and characterization of several phosphate starvation induced genes in tomato. Several manuscripts have been published as a part of BARD supported research activity. Furthermore two important publications capturing the key findings of our research are ready for publication.

This project helped us train three postdoctoral research associates and several graduate students including two doctoral students at Purdue University. The genetic and molecular resources developed during this project will help us better understand plant responses to phosphate deficiency and generate phosphate efficient plants. Altered morphology of tomato plants over-expressing the phosphor-protein phosphatase could be

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utilized to extend flowering with a potential to increase fruit yield. Highlights of the some of the recent research outcomes are presented below (See **Recent Research Highlights**).

Details of cooperation: whether and how project objectives were promoted as a result of the cooperation.

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List of Publications: Include only reviewed publications reporting on work at least partially supported by BARD and which includes an acknowledgement to BARD.

Raghothama KG 2005. Phosphorus and plant nutrition: An overview. In "Phosphorus: Agriculture and the Environment" a monograph from the American Society of Agronomy-Crop science Society of America and Soil Science Society of America. pp 355-378

Jain A, Cao A, Karthikeyan AS, Baldwin JC and Raghothama KG. 2005. **Phosphate deficiency suppresses expression of light-regulated psbO and psbP genes encoding extrinsic proteins of oxygen-evolving complex of PSII.** *Curr Sci.* 89: 1592-1596.

Raghothama KG 2005. Phosphorus. In Plant Nutritional Genomics. Eds. Martin Broadley, and Philip White, Blackwell Publishing pp112-126.

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Raghothama KG, Karthikeyan AS 2005. Phosphate acquisition. Plant Soil 274 : 37-49

Ajay Jain, Maria Jose Vasconcelos, Shivendra V. Sahi* and K.G. Raghothama. 2005. Molecular Mechanisms of Plant Adaptation to Phosphate Deficiency (Accepted pending final revision for Plant Breeding Reviews)

Guo-hua Xu^a, Veronique Chague^b, Cathy Melamed-Bessudo^b, Yoram Kapulnik^c, Ajay Jain^d Kashchandra.G. Raghothama^d, Avraham A. Levy^b, Avner Silber^{e,1} 2005. Isolation and Functional Characterization of LePT4, a Mycorrhizal Enhanced Phosphate Transporter in Tomato. (under revision).

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Highlights of recent research findings

Table 1. Mycorrhizal fungi infection effects on fresh weights of shoot, root and root/shoot (Rt/Sht) ratio in w.t. and *lept4* grown under deficient (0.05 mM) or normal (1mM) Pi concentrations. w.t: wild type; *lept4*: *lept4* mutant; -Myc and +Myc: without and with the mycorrhizae inoculum, respectively.

Treatments	0.05 mM Pi			1.0 mM Pi		
	Shoot	Root	Rt/Sht	Shoot	Root	Rt/Sht
	g/plant FW			g/plant FW		
w.t -Myc	5.7	2.4	0.42	26.8	8.6	0.32
w.t +Myc	9.0	4.1	0.46	28.5	8.9	0.31
<i>lept4</i> -Myc	3.7	1.6	0.43	22.4	7.2	0.32
<i>lept4</i> +Myc	5.3	2.1	0.39	23.9	7.5	0.32
Probability > F						
Plant	<0.0001	<0.0001	0.3121	0.0007	0.001	0.9960
Myc	0.0004	0.0004	0.9686	0.1401	0.3498	0.4313
Plant*Myc	0.112	0.1322	0.1300	0.9460	0.9791	0.9651
LSD _{0.05}	2.08	1.06	0.099	4.18	1.38	0.036

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Figure 1: Responses of Micro-Tom w.t. and *lept4* mutant to mycorrhizal infection during growth under Pi normal (1 mM) and deficient (0.05 mM) conditions. +Myc represents sand-based mycorrhizal inoculum containing *Glomus intraradices*, whereas -Myc indicates control plants which were not infected with mycorrhizal fungi. The *lept4* mutant showed a significant decrease in growth under low P conditions with or without mycorrhizal fungi infection.

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Figure 2: Expression of phosphate transporters in tomato. Northern blot analysis of tomato phosphate transporter transcripts in Micro-Tom w.t and in the *lept4* mutant grown under P + (continuous supply of 0.25-mM P for 35 days) and P- (initial supply of 0.25-mM P for 28 d followed by Pi starvation for next 7 d) conditions. Equivalence of RNA loading in all the lanes is indicated by ethidium bromide-stained rRNA (bottom panel).

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WT

Sense

Anti-Sen

Figure 3: Effect of altered expression of LePS2 gene coding for a protein phosphatase in tomato. Growth of LePS2 gene over-expressing plants (sense) was significantly delayed as compared to that of wild type plants. The leaves of sense plants were darker in color and smaller in size. Overall there was a delay in flowering of sense plants. These data suggests that the protein phosphatase LePS2 has a significant effect on plant growth and flowering.

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Fig 5 Plant height, shoot fresh weight and total leaf area of tomato transgenic plants over expressing *LePS2:1* gene. Tomato plants were grown in soil for 5 weeks after germination. The plants were supplied with fertilizer water every 3 days. Leaf area was measured by using the L1-3100 L1-COR . WT: wild type. A10: antisense plant. S3: sense plant.