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BARD Final Scientific Report
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Date of Submission of the report: January 20, 2001

BARD Project Number: IS-2972-98

Project Title: Identification of citrus fruit-specific promoters and their use in molecular engineering

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.
Microprojectile bombardment, Transformation

Abbreviations commonly used in the report, in alphabetical order:

Budget: IS: \$ 45,000

US: \$ 30,000

Total: \$ 75,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

630.72

BAR-108

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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				
Submitted, in review, in preparation				
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts			1	1
Not refereed (proceedings, reports, etc.)			1	1

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				None
Longer Visits (Sabbaticals)				None

Description Cooperation:

This current one year BARD project was funded to assist in developing assays to monitor gene expression in citrus fruit. The Israeli side examined the use of microprojectile bombardment as a tool for transient gene expression analysis in various citrus fruit tissues, and the US side optimized the technical parameters required for *Agrobacterium*-mediated transformation of citrus. In addition, both sides made progress in isolating citrus fruit-specific promoters. The cooperation was by dividing the research tasks between both labs.

Patent Summary (numbers)

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

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1. Abstract

This one year BARD project was funded to develop methods to monitor promoter activity and gene expression patterns in citrus fruit. To fulfill this goal, we divided the research tasks between both labs so that the Israeli side evaluated the use of microprojectile bombardment as a tool to evaluate transient gene expression in various citrus fruit tissues, and the US side optimized technical parameters required for *Agrobacterium*-mediated transformation of various citrus cultivars.

Microprojectile bombardment appeared to be a very efficient method for transient gene expression analysis in citrus leaf tissues but was somewhat less applicable in fruit tissues. Nevertheless, we did succeed to achieve significant levels of 35S-GUS gene expression in young green flavedo tissue. However, only single random spots of 35S-GUS gene expression were detected mature flavedo and in juice sacs and albedo tissue. Overall, we assume that following some more technical improvements particle bombardment could provide a useful technique to rapidly analyze promoter activity at least in the flavedo tissue.

For *Agrobacterium*-mediated transformation, we found that shoot cultures of 'Washington' navel oranges, 'Fairchild' mandarins, 'Eureca' lemons, 'Troyer' citrange and various grapefruits provided a more reliable and consistent source of tissue for transformation than germinated seedlings. Moreover, various growth media's (McCown, Quoirin & Lepoivre, DCR) further improved shoot and root growth relative to MS mineral media, which is commonly used. Also pure white light (using bulbs which do not emit UV or blue light) improved shoot growth in various citrus varieties, and paromomycin appeared to be a more efficient antibiotic for the selection of transgenic plants than Kanamycin. Overall, these optimizations improve transformation efficacy and shoot growth and rooting capacity.

In addition to the development of transformation methods, both Israeli and US labs achieved progress in the identification of citrus fruit-specific promoters. In Israel, we isolated a 3.6 kb promoter fragment of the thiamine biosynthesis *c-thi* gene, which is highly expressed in fruit peel tissue, whereas in the US we isolated a 1.5 kb promoter fragment of the citrus seed-specific cDNA *CssH*. The identification of more fruit-specific cDNAs and their corresponding promoter regions is currently in progress.

2. Achievements

Significance of the main scientific achievements

This current one year BARD project was funded to assist in developing methods to monitor promoter activity and gene expression patterns in citrus fruit. To fulfill this goal, we divided the research tasks between both labs so that the Israeli side evaluated the use of microprojectile bombardment as a tool for transient gene expression analysis in various citrus fruit tissues, and the US side optimized technical parameters required for *Agrobacterium*-mediated transformation of citrus. Microprojectile bombardment provides a rapid method to monitor promoter activity following transient gene expression, without needing to construct transgenic plants. On the other hand, production of transgenic plants is a laborious process, but is the most reliable method to evaluate promoter activity in plant tissues and in any case is essential for genetic engineering and molecular breeding.

We found that Microprojectile bombardment was indeed a very efficient method to monitor promoter activity in citrus leaf tissues, but seemed to be much less applicable in citrus fruit. The difficulties in transforming and expressing foreign DNA in citrus fruit tissues may be explained by their relative low acidic pH, and by the difference in their cell structure and perhaps the cell wall barriers present upon the flavedo. Nevertheless, we had succeeded to achieve significant levels of 35S-GUS gene expression following microprojectile bombardment in young green flavedo tissue. However, only single random spots of 35S-GUS gene expression were detected following microprojectile bombardment of mature flavedo or in juice sacs and in the albedo. The optimal technical conditions required for microprojectile bombardment of the flavedo tissue consisted using 1,550 psi rupture disks and a vacuum of 26 inches Hg within the chamber. The bombarded tissue should be placed at a distance of 10 cm from the biolistic microcarriers and each sample was transformed four consecutive times. Insertion of two rings into the microcarrier launch also significantly improved transformation efficiency. Finally, we recommend using larger M-25 tungsten particles rather than M-10 particles. Overall, we assume that following some more technical improvements, particle bombardment could provide a useful technique to rapidly analyze promoter activity at least in young flavedo tissue.

For *Agrobacterium*-mediated transformation of various citrus cultivars, we found that optimization of some basic parameters may clearly improve transformation efficacy. For example, we found that using shoot cultures of 'Washington' navel oranges, 'Fairchild'

mandarins, 'Eureca' lemons, 'Troyer' citrange and various grapefruits provided a more reliable and consistent source of tissue for transformation than germinated seedlings. Moreover, three different growth media's (McCown, Quoirin & Lepoivre, DCR) showed greatly improved shoot and root growth capacities relative to MS mineral media, which is currently used. Also light quality appeared to be important for shoot growth, and pure white light (using bulbs which do not emit UV or blue light) clearly improved shoot growth in various citrus varieties. Another improvement is that paromomycin appeared to be a much more efficient antibiotic than Kanamycin for the selection of the transgenic plants. Overall, these above optimizations improve transformation efficacy and shoot growth and rooting in tissue culture conditions.

In addition to the development of transformation methods, both Israeli and US labs made progress in the identification of citrus fruit-specific promoters. In Israel, Dr. Holland isolated by screening a citrus genomic library a 3.6 kb promoter fragment of the 'Valencia' thiamine biosynthesis *c-thi* gene, which is highly expressed in fruit peel tissue during maturation. In the US, Dr. Walling isolated by a PCR-based method of differential display twenty putative citrus seed-specific cDNA inserts. Five of these clones were sequenced and their expression patterns were evaluated by RT-PCR, using gene specific primers. It was found, that only the *CssH* cDNA appeared to be seed-specific, and by using the Genome Walker strategy a 1.5 kb fragment of its promoter region was isolated. The identification and isolation of more fruit-specific cDNAs and their corresponding promoter regions are currently in progress.

Agriculture and/or economic impacts of the research findings

Identification of promoters that are expressed in specific fruit tissues, such as the peel, juice or seeds, will facilitate future improvement in fruit quality by enabling to direct the expression of specific genes to specific fruit tissues by genetic engineering strategies. In this research project, we evaluated methods to monitor promoter activity in citrus fruit tissues in order to be able to isolate and characterize such commercially important-fruit-specific promoters. The results of the current research allows the use of microprojectile bombardment as a rapid assay to monitor promoter activity in the flavedo tissue following transient expression.

Moreover, we hereby optimized the technical parameters required for *Agrobacterium*-mediated transformation of citrus cultivars and, thus, overall improved transformation efficacy and shoot and rooting growth in tissue culture conditions.

Details of cooperation

To fulfill the goals of the current one year BARD project, we conducted a supportive mode of cooperation in which we divided the research tasks between both sides. The Israeli side focused on monitoring promoter activity following microprojectile bombardment and the US side focused on optimizing basic protocols required for *Agrobacterium*-mediated transformation of citrus. Both sides were also interested in identifying fruit-specific promoters, and the Israeli side focused on the isolation of a promoter from a peel-specific gene, whereas the US side focused on the isolation of promoters from seed-specific genes.

Before the initiation of the research project, Dr. D. Holland, the collaborating investigator from the Israeli side, has visited Dr. L. Walling lab at UC Riverside to discuss the research tasks. During the funded research period both sides maintained an ordinary line of communication by E-mail.

List of Publications

Porat, R., Avidan, N. and D. Holland (2000). Microprojectile bombardment of DNA into citrus leaf and fruit tissues. Proceedings of the International Society of Citriculture (In press).