25

BARD

FINAL REPORT

PROJECT NO. IS-3333-02

**Genomic Approach to the Improvement of Fruit
Quality in Melon (*Cucumis melo*) and Related
Cucurbit Crops**

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**BARD Research Grant
Final Scientific Report
Cover Page**

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**Project Title: Genomic approach to the improvement of fruit quality in melon
(Cucumis melo) and related cucurbit crops**

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

Melon database; microarray analysis

Abbreviations commonly used in the report, in alphabetical order:

ESTs – expressed sequence tags

ICuGI – international cucurbit genomics initiative

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Signature

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BARD Research Grant Final Scientific Report

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Cooperation Summary (numbers)

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Description Cooperation:

The collaboration was excellent and according to the original proposal: melon genetic material, RNA and expression libraries were developed by the Israeli team and were transferred to the US. Sequencing, database as well as macro- and microarrays were developed by the US team. Sequence and database information has been transferred to Israel immediately upon development. Macroarray analysis was performed in Israel while microarray analysis has begun in the US using RNA sent from Israel and will continue in both locations. The PIs met every year in conferences and discussed ongoing issues. The last meeting was at the Plant and animal genome meeting (Jan. 2006, San Diego) leading to the selection of the database to be the ICuGI central database.

Final Report IS 3333-02: Genomic approach to the improvement of fruit quality in melon (*Cucumis melo*) and related cucurbit crops

Abstract

Fruit quality is determined by numerous genetic traits that affect taste, aroma, texture, pigmentation, nutritional value and duration of shelf-life. The molecular basis of many of these important traits is poorly understood and its understanding offers an excellent opportunity for adding value to agricultural products. Improvement of melon fruit quality was the primary goal of the project.

The original objectives of the project were:

1. The isolation of a minimum of 1000 fruit specific ESTs.
2. The development of a microarray of melon fruit ESTs.
3. The analysis of gene expression in melon using melon and tomato fruit enriched microarrays.
4. A comprehensive study of fruit gene expression of the major cucurbit crops.

In our current project we have focused on the development of genomics tools for the enhancement of melon research with an emphasis on fruit, specifically the first public melon EST collection. We have also developed a database to relay this information to the research community and developed a publicly available microarray. The release of this information was one of the catalysts for the establishment of the International Cucurbit Genomic Initiative (ICuGI, Barcelona, Spain, July 2005) aimed at collecting and generating up to 100,000 melon EST sequences in 2006, leveraging a significant expansion of melon genomic resources.

A total of 1000 ESTs were promised under the original proposal (Objective 1). Non-subtracted mature fruit and young fruit flesh of a climacteric variety in addition to a non-climacteric variety resulted in the majority of additional EST sequences for a total of 4800 attempted reads. 3731 high quality sequences from independent ESTs were assembled, representing 2,467 melon unigenes (1,873 singletons, 594 contigs). In comparison, as of June 2004, a total of 170 melon mRNA sequences had been deposited in GENBANK. The current project has thus resulted in nearly five-fold the number of ESTs promised and ca. 15-fold increase in the depth of publicly available melon gene sequences. All of these sequences have been deposited in GENBANK and are also available and searchable via multiple approaches in the public database (<http://melon.bti.cornell.edu>). Our database was selected as the central location for presentation of public melon EST data of the International Cucurbit Genomic Initiative.

With the available unigenes we recently constructed a microarray, which was successfully applied in hybridizations (planned public release by August 2006). Current gene expression analyses focus on fruit development and on comparative studies between climacteric and non-climacteric melons. Earlier, expression profiling was conducted using macroarrays developed at the preliminary stage of the project. This analysis replaced the study of tomato microarray following the recommendations of the reviewers and the panel of the original project.

Comparative study between melon and other cucurbit crops have begun, mainly with watermelon, in collaboration with Dr. Amnon Levi (USDA-ARS).

In conclusion, all four objectives have been addressed and achieved. In the continuation project that have been approved we plan to apply the genomic tools developed here to achieve detailed functional analyses of genes associated with major metabolic pathways related to fruit development and the transcription factors regulating them.

Final Report IS 3333-02: Genomic approach to the improvement of fruit quality in melon (*Cucumis melo*) and related cucurbit crops

Achievements

1. The Major achievements of the project were:

1.1 ESTs and database.

In our current project we focused on the development of genomics tools for the enhancement of melon research with an emphasis on fruit, specifically the first public melon EST collection. We also developed a database (<http://melon.bti.cornell.edu>) to relay this information to the research community. The release of this information was one of the catalysts for the establishment of the International Cucurbit Genomic Initiative (ICuGI, Barcelona, Spain, July 2005) aimed at collecting and generating up to 100,000 melon EST sequences in 2006, leveraging a significant expansion of melon genomic resources. Our database was selected to be the major database of this initiative.

Melon fruit ESTs were derived from 20 independent libraries (Appendix Table 1) constructed under the current project, including subtracted libraries between fruit and vegetative tissues and between various genotypes that differ in fruit quality traits (including sugar content, pH and aroma). A total of 1000 ESTs were promised under the original proposal. Non-subtracted mature fruit and young fruit flesh of a climacteric variety in addition to a non-climacteric variety resulted in the majority of additional EST sequences for a total of 4800 attempted reads. 3,731 high quality sequences from independent ESTs were assembled representing 2467 melon unigenes (1873 singletons, 594 contigs). In comparison, as of June 2004, a total of 170 melon mRNA sequences had been deposited in GENBANK. The current project thus resulted in nearly five times the number of ESTs promised and ca. 15-fold increase in the depth of publicly available melon gene sequences. All of these sequences have been deposited in GENBANK and are also available and searchable via multiple approaches in the public database (<http://melon.bti.cornell.edu>).

We obtained additional 1800 melon ESTs from a colleague (Prof. S. Wolf, Hebrew University, Israel) in addition to requests to contribute by others. The total current number of ESTs in the database is 5649, representing 3,254 unigenes (845 contigs and 2409 singletons).

1.2 Microarray development and application

With the available unigenes we recently constructed a microarray that we successfully applied it in test hybridizations (planned public release by August 2006). We

currently utilize this array to characterize the transcriptom activity in distinct stages of fruit development, in climacteric and non-climacteric melons (Appendix: Fig. 1). Earlier, a sub-cluster of ca 5000 clones obtained from four of our subtraction libraries were printed on nylon membranes (macroarrays) that were applied to gain knowledge of candidate genes associated with fruit development in melon (see appendix, Yariv et al. in preparation).

Under our project continuation, we will use this array in combination with targeted metabolite profiling, to explore fruit gene expression in unique melon genotypes (including transgenic ripening mutants and near isogenic lines); in order to more accurately identify candidates for functional analysis. This analysis will focus on the identification of genes belonging to important fruit metabolic pathways (sugar, organic acids and aroma volatiles) as well as on transcription factors which may control these pathways or participate in broader regulation of ripening.

1.3 Data mining of the EST database and functional analysis.

Data mining of the database identified candidate genes participating in major metabolic pathways. Functional analyses of genes that are aroma-related have begun in collaboration with Dr. Efraim Lewinsohn, (see appendix: Ibdah et al 2006 and Benyamini et al. in preparation). In addition, recent creation of a transgenic melon with extended shelf-life (see appendix: Fig. 2) demonstrates the potential of our approach and provides an attractive genetic background and tool to select candidate genes for manipulation. The comparative study of major genes of additional cucurbit crops, mainly watermelon, with additional cucurbit crops have also begun.

2. Agricultural impacts of the research findings

An example for an immediate impact of the tools developed in this project is the development of molecular markers. Over 100 SSR markers and over 60 SNP markers have been developed by us and are in the process of locating them on the melon map. All these markers were derived from genes associated with fruit ripening and quality and therefore their application is expected to have an immediate benefit for melon breeding. Recently, the information concerning SSR motives has been added to the database and is therefore publicly available.

3. Details of cooperation

The collaboration was excellent and according to the original proposal: melon genetic material, RNA and expression libraries were developed by the Israeli team and were transferred to the US. The sequencing and the database, as well as macro- and microarrays, were developed by the US team. Sequence and database information were transferred to Israel immediately upon development. Macroarray analysis was performed in Israel while microarray analysis has begun in the US using RNA sent from Israel and will continue in both locations. The PIs met every year in conferences and discussed ongoing issues. The last meeting was at the Plant and animal genome meeting (Jan. 2006, San Diego) leading to the selection of the database to be the ICuGI central database.

4. List of publications (in reviewed journals)

Giovannoni J. (2004) Genetic regulation of fruit development and ripening. *The Plant Cell*. 16: S170 -180.

Adams-Phillips, L., Barry, C. and Giovannoni, J. (2004) Signal transduction systems regulating fruit ripening. *Trends in Plant Science*. 9: 331-338

Alba, R., Fei, Z., Payton, P., Liu, Y., Moore, S., Debbie, P., Gordon, J., Rose, J., Martin, G., Tanksley, S., Bouzayen, M., Jahn, M., and Giovannoni, J. (2004) ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. *Plant Journal*. 39:697-714.

Ibdah, M., Azulay, Y., Portnoy, V., Wasserman, B., Bar, E., Meir, A., Burger, Y., Hirschberg, J., Schaffer, A.A., Katzir, N., Tadmor, Y., and Lewinsohn, E. (2006). Functional characterization of CmCCD1, a carotenoid cleavage dioxygenase from melon. *Phytochemistry* (in press).

Burger, Y., U. Sa'ar, H. S. Paris, E. Lewinsohn, N. Katzir, Y. Tadmor and Schaffer A. A. (2006). Genetic variability as a source of new valuable fruit quality traits in *Cucumis melo*. *Israel Jour. of Plant Sci.* (in press).

Appendix

Final Report IS 3333-02: Genomic approach to the improvement of fruit quality in melon (*Cucumis melo*) and related cucurbit crops

Table of contents	Page
Table 1: list of cDNA libraries	1
Figure 1: Melon microarray	3
Figure 2: Transgenic melon RIN-RNAI	4
Publications :	
• Giovannoni et al. 2004	5
• Adams-Phillips et al. 2004	16
• Alba et al. 2004	24
• Ibdah et al. 2006	42
• Burger et al. In press	53
• Katzir et al. Cucurbitaceae 2006	74
• Yariv et al. In preparation	82

Table1. A list of Melon cDNA libraries constructed as part of the current BARD project

	library name	cultivar	tissue	Tester (SSH)	Driver (SSH)
1	Melon subtraction library*	Noy Yizre'el	fruit (mixed stages)	Noy Yizre'el	Vedrantais
2	Melon subtraction library*	Vedrantais	fruit (mixed stages)	Vedrantais	Noy Yizre'el
3	Melon subtraction library*	Noy Yizre'el	roots, stems and leaves	plant	fruit
4	Melon subtraction library*	Noy Yizre'el	fruit (mixed stages)	fruit	plant
5	Melon subtraction library*	Dulce	fruit (mixed stages)	Dulce	PI414723
6	Melon subtraction library*	PI414723	fruit (mixed stages)	PI414723	Dulce
7	Melon subtraction library*	Rochet	fruit (mixed stages)	Rochet	Noy Yizre'el
8	Melon subtraction library*	Noy Yizre'el	fruit (mixed stages)	Noy Yizre'el	Rochet
9	Melon subtraction library*	Dulce	fruit (mixed stages)	Dulce	Noy Yizre'el
10	Melon subtraction library*	Faggous	fruit (mixed stages)	Faggous	Noy Yizre'el
11	Melon subtraction library*	Noy Yizre'el	fruit (mixed stages)	Noy Yizre'el	Faggous
12	Melon subtraction library*	Noy Yizre'el	Mature flesh (47 DPA)	Mature flesh (47 DPA)	Young flesh (25 DPA)
13	Melon subtraction library*	Noy Yizre'el	Young flesh (25 DPA)	Young flesh (25 DPA)	Mature flesh (47 DPA)
14	Melon subtraction library*	Noy Yizre'el	Mature rind (47 DPA)	Mature rind (47 DPA)	Young rind (25 DPA)
15	Melon subtraction library*	Noy Yizre'el	Young rind (25 DPA)	Young rind (25 DPA)	Mature rind (47 DPA)
16	Melon subtraction library*	F2 Noy Yizre'el and Faggous	Mature fruit	High sucrose	Low sucrose
17	Melon subtraction library*	F2 Noy Yizre'el and Faggous	Mature fruit	Low sucrose	High sucrose
18	Melon carotenoid or organic acid metabolism related gene library**	Noy Yizre'el	fruit	N/A	N/A
19	Melon sugar metabolism related gene library**	Noy Yizre'el	fruit	N/A	N/A
20	Melon mature fruit library***	Noy Yizre'el	a mixture of mature green fruits and mature yellow fruits	N/A	N/A
21	Melon young fruit library***	Noy Yizre'el	a mixture of fruits at 0, 1, 3, 12, and 25 DPA	N/A	N/A
23	DUL mature fruit library***	Dulce	mature fruit	N/A	N/A
24	TAD mature fruit library***	Tam Dew	mature fruit	N/A	N/A

- * Library constructed using Clontech PCR-Select cDNA Subtraction kit and the pGEM-T easy vector
- ** Partial sequences, cloned based on homology to conserved sequences with the pGEM-T easy vector
- *** Library constructed using Stratagene ZAP cDNA Synthesis Kit and the pBK-CMV vector

Fig.1

Melon cDNA microarray

The array will contain 2387 unique elements and each element will be printed in duplicate on the array

The Melon cDNA array and protocols will be publicly available soon and distributed through the Center for Gene Expression Profiling (<http://bti.cornell.edu/CGEP/CGEP.html>)

Fig. 2: Transgenic melon-RIN RNAi cantaloupe melons with altered ripening properties. All three melons were harvest when the fruit completely separated from the vine, which corresponded to 36 DAP. **A.** Melon-RIN RNAi line HBJI12b-green flesh at maturity, firmer yet aromatic. **B.** Non-transformed control, flesh loose and watery. **C.** Melon-RIN line HBJI4 - firm flesh, aromatic, just a bit of water soaking.