Cover Page

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Project Title:
Molecular studies of postharvest leaf and flower abscission

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Keywords not appearing in the title and in order of importance. Avoid abbreviations.
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Petiole, Postharvest quality, Tomato, Transcriptome

Abbreviations commonly used in the report, in alphabetical order: See page 2

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution
Molecular Studies of Postharvest Leaf and Flower Abscission
BARD Research project IS-3815-05

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List of Abbreviations Used in the Report

ACC = 1-amino-cyclopropane-1-carboxylic acid
ACO = ACC oxidase
ACS = ACC synthase
ARF = auxin responsive factor
AZ = abscission zone
CEL = cellulose
CHS = chalcone synthase
ERF = ethylene responsive factor
EST = expressed sequence tag
EXP = expansin
IAA = indole-3-acetic acid
LHC = light harvesting complex
LOX = lipoygenase
1-MCP = 1-methylcyclopropene
NAZ = non abscission zone
NPA = N-1-naphthylphthalamic acid
PDS = phytoene desaturase
PG = polygalacturonase
PK = protein kinase
SAM = S-adenosyl methionine
TAPG = tomato abscission polygalacturoanse
TC = tentative consensus sequence
TF = transcription factor
TIGR = The Institute for Genomic Research
TRV = tobacco rattle virus
VIGS = virus-induced gene silencing
A. Overall Summary and Statistics

A.1. Publication Summary (numbers)

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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.

Cooperation Summary (numbers)

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Description of Cooperation:

As this one-year project was a feasibility study, the two objectives were divided between the two collaborating teams according to their expertise. Thus, the American team developed the VIGS tool, while the Israeli team performed the microarray analysis. The two research teams were linked via intensive e-mail correspondence, and also met twice for fruitful discussions related to the project and writing of this report: Prof. Reid visited in Israel in May 2006 and met with the Israeli team, and all the five collaborators involved in the project have met in the US, at a GRC meeting in July 2006.

Patent Summary (numbers)

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A.2. Abstract

Original objectives: Understanding the regulation of abscission competence by exploring the nature and function of auxin-related gene expression changes in the leaf and pedicel AZs of tomato (as a model system), was the main goal of the previously submitted proposal. We proposed to achieve this goal by using microarray GeneChip analysis, to identify potential target genes for functional analysis by virus-induced gene silencing (VIGS). To increase the potential of accomplishing the objectives of the previously submitted proposal, we were asked by BARD to show feasibility for the use of these two modern techniques in our abscission system. Thus, the following new objectives were outlined for the one-year feasibility study: 1. to demonstrate the feasibility of the VIGS system in tomato to perform functional analysis of known abscission-related genes; 2. to demonstrate that by using microarray analysis we can identify target genes for further VIGS functional analysis.

Background to the topic: It is a generally accepted model that auxin flux through the abscission zone (AZ) prevents organ abscission by rendering the AZ insensitive to ethylene. However, the molecular mechanisms responsible for acquisition of abscission competence and the way in which the auxin gradient modulates it are still unknown. Understanding this basic stage of the abscission process may provide us with future tools to control abscission for agricultural applications. Based on our previous study, performed to investigate the molecular changes occurring in leaf and stem AZs of Mirabilis Jalapa L., we have expanded our research to tomato, using genomic approaches that include modern techniques for gene discovery and functional gene characterization. In our one-year feasibility study, the US team has established a useful system for VIGS in tomato, using vectors based on the tobacco rattle virus (TRV), a Lc reporter gene for silencing (involved in regulation of anthocyanin biosynthesis), and the gene of interest. In parallel, the Israeli team has used the newly released Affymetrix Tomato GeneChip to measure gene expression in AZ and non-AZ tissues at various time points after flower removal, when increased sensitivity to ethylene is acquired prior to abscission (at 0-8 h), and during pedicel abscission (at 14 h). In addition, gene expression was measured in the pedicel AZ pretreated with the ethylene action inhibitor, 1-methylcyclopropene (1-MCP) before flower removal, to block any direct effects of ethylene.

Major conclusions, solutions and achievements: 1) The feasibility study unequivocally established that VIGS is an ideal tool for testing the function of genes with putative roles in abscission; 2) The newly released Affymetrix Tomato GeneChip was found to be an excellent tool to identify AZ genes possibly involved in regulation and execution of abscission. The VIGS-based study allowed us to show that TAPG, a polygalacturonase specifically associated with the tomato AZ, is a key enzyme in the abscission process. Using the newly released Affymetrix Tomato GeneChip we have identified potential abscission regulatory genes as well as new AZ-specific genes, the expression of which was modified after flower removal. These include: members of the Aux/IAA gene family, ethylene signal transduction-related genes, early and late expressed transcription factors, genes which encode post-translational regulators whose expression was modified specifically in the AZ, and many additional novel AZ-specific genes which were previously not associated with abscission. This microarray analysis allowed us to select an initial set of target genes for further functional analysis by VIGS.

Implications: Our success in achieving the two objectives of this feasibility study provides us with a solid basis for further research outlined in the original proposal. This will significantly increase the probability of success of a full 3-year project. Additionally, our feasibility study yielded highly innovative results, as they represent the first direct demonstration of the functional involvement of a TAPG in abscission, and the first microarray analysis of the abscission process. Using these approaches we could identify a large number of genes involved in abscission regulation, initiation and execution, and in auxin-ethylene cross-talk, which are of great importance, and could enable their potential functional analysis by VIGS.
A.3. Achievements

A.3.1. Significance of main scientific achievements or innovations

BARD recommended objectives for the one year feasibility study, which reflected the concerns of the proposal reviewers and the panel, were: 1. to demonstrate the feasibility of the VIGS system in tomato to perform functional analysis of known abscission-related genes; 2. to demonstrate that by using microarray analysis we can identify target genes for further VIGS functional analysis to achieve the objectives of the original proposal. Success in achievement of these two objectives should provide a solid basis for further research as outlined in the objectives of the original proposal. We believe that we have successfully fulfilled, and even more than that, the two objectives of the feasibility study, and by this we have significantly increased the probability of success of a full 3-year project. Thus, we have established useful tools for elucidation of the unknown early molecular regulatory events controlling the initiation of abscission, as the AZ becomes sensitized to ethylene.

The major conclusions and achievements of this feasibility study are: 1. We established unequivocally that, in our chosen model plant tomato, VIGS is an effective and fast tool for testing the function of genes with putative roles in abscission; 2. We have used successfully the newly released Affymetrix Tomato GeneChip for identification of AZ-associated genes involved in regulation or execution of the abscission process, which will serve for further functional analysis by VIGS.

Using VIGS, we demonstrated that TAPG, a PG specifically associated with the tomato AZ, is a key enzyme in the abscission process. This conclusion was based on the observed retardation of petiole abscission in the TAPG-silenced plants, and on the increase in the force required for petiole separation. It should be noted that this is the first clear demonstration of the functional involvement of TAPG in the abscission process. Previous studies pointing to a role for PGs and expansins, have largely been based on correlations between abscission events and abundance of transcripts or activity of the encoded enzymes. However, the role of two cellulases in floral and fruit abscission was demonstrated by means of antisense suppression.

Using the newly released Affymetrix GeneChip we have identified potential abscission regulatory genes as well as many new AZ-specific genes, the expression of which was modified after flower removal. These include: members of the Aux/IAA gene family, ethylene-related genes involved in the signal transduction and biosynthesis pathways; early and late expressed TF's, genes which encode post-translational regulators whose expression was modified specifically in the AZ, and many additional novel AZ-specific genes which previously were not associated with abscission. This microarray analysis allowed us to select
an initial set of target genes for further functional analysis by VIGS. Apart of the identified genes with possible regulatory functions, we have also identified novel AZ-specific genes that might function in the later mechanistic stages of the abscission process. These include novel AZ-specific genes encoding for cell wall degrading enzymes, taking part in cell separation, generation of protective layer and defense.

We believe that our results are highly innovative, as they represent the first microarray analysis of the abscission process. Using this approach we could identify a large number of genes involved in abscission regulation, initiation and execution and in auxin-ethylene cross-talk, which are of great importance. Taken together, it can be surely concluded from these experiments that the newly released Affymetrix Tomato GeneChip is an excellent tool for identification of AZ-related genes and transcriptome analysis, and that the developed VIGS system in tomato is an excellent technique for analyzing their functions. All this provides us with enough preliminary results as a promising start for a full project.

**A.3.2. Details of cooperation**

As this one-year project was a feasibility study, the two objectives were divided between the two collaborating teams according to their expertise. Thus, the American team developed the VIGS tool, while the Israeli team performed the microarray analysis. The two research teams were linked via intensive e-mail correspondence, and also met twice for fruitful discussions related to the project and the writing of this report: Prof. Reid visited in Israel in May 2006 and met with the Israeli team, and all the five collaborators involved in the project have met in the US, at a GRC meeting in July 2006.

We are planning now to start the functional analysis of target genes found in the microarray experiment (Israel), using the VIGS technique established in this project (USA).

**A.3.3. List of publications**


B. Appendix

This one year research has been approved for funding as a feasibility study. The original goal of the proposal was to understand the regulation of abscission competence by exploring the nature and function of auxin-related gene expression changes in the leaf and pedicel AZs of tomato (as a model system). Part of our specific objectives were: 1) to identify, using microarray analysis, genes whose expression in the tomato leaf and pedicel AZs changes in response to auxin depletion; and 2) to examine the effect of silencing such genes on ethylene sensitivity and abscission competence of the leaf and pedicel AZs, and on the AZ transcriptome.

The feasibility study gave us the opportunity to demonstrate the usefulness and effectiveness of the VIGS functional analysis system in tomato using known abscission-related genes. Another main concern of the proposal reviewers and the panel was related to the fact that the commercial available TOM1 microarray chip (proposed to be used in this research) was not enriched for AZ-specific genes, and, therefore, might not provide a reliable source for the identification and isolation of abscission-related genes.

Regarding this, the objectives of our one-year feasibility study were defined as follows: 1. To demonstrate the feasibility of the VIGS functional analysis system in tomato using known abscission-related genes; 2. To demonstrate that by using microarray GeneChips we can identify target genes for further VIGS functional analysis, to achieve the objectives of the original proposal. Fortunately, as we initiated this research, the new Affymetrix Tomato GeneChip was released, and we could use it to successfully fulfill this objective.

The results of the feasibility study summarized in this report are divided into two separate chapters, according to the two objectives described above.