Final Scientific Report

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations. Abscission zone, auxin depletion, ethylene, hormone cross-talk, 1-methyl-cyclopropene, petiole, pedicel, signal transduction, tomato, transcription factors, microarray, virus-induced gene silencing

Abbreviations commonly used in the report, in alphabetical order:

ACC = 1-amino-cyclopropane-1-carboxylic acid; ACO = ACC oxidase; ACS = ACC synthase; ARF = auxin responsive factor; AZ = abscission zone; Cel = cellulase; ERF = ethylene responsive factor; ETR = ethylene resistant; FAZ = flower AZ; FR = flower removal; IAA = indole-3-acetic acid; LAZ = leaf AZ; LRR = Leu-rich repeat; 1-MCP = 1-methylcyclopropene; NAZ = non-AZ; NPA = *N*-1-naphthylphthalamic acid; PR = pathogen-related; ROS = reactive oxygen species; RT-qPCR = real time quantitative PCR; SAM = S-adenosyl methionine; TAPG = tomato abscission polygalacturonase; TF = transcription factor; TIGR = The Institute for Genomic Research; TRV = tobacco rattle virus; VIGS = virus-induced gene silencing; XET = xyloglucan endohydrolase endotransglycosylase.

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US: 129,000\$

Total: 290,000\$

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Molecular Studies of Postharvest Leaf and Flower Abscission

BARD Project Number: **IS-4073-11C**

Final Report

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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	3	1		4
Submitted, in review, in preparation	1			1
Invited review papers				
Book chapters		1		1
Books				
Master theses			1	1
Ph.D. theses				
Abstracts	3			
Not refereed (proceedings, reports, etc.)	2			2

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	3	4
Longer Visits (Sabbaticals)				

Description of Cooperation:

Both synergistic and complementary modes of cooperation were employed: Each research group has contributed its specific expertise in techniques and scientific knowledge for studying and elucidating the early regulatory molecular events underlying organ abscission in tomato plants, used as a model system.

Patent Summary (numbers)

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

Abstract

Original objectives: To understand the regulation of abscission by exploring the nature of changes of auxin-related gene expression in tomato (*Lycopersicon esculatum* Mill) abscission zones (AZs) following organ removal, and by analyzing the function of these genes. Our specific goals were: 1) To complete the microarray analyses in tomato flower and leaf AZs, for identifying genes whose expression changes early in response to auxin depletion; 2) To examine, using virus-induced gene silencing (VIGS), the effect of silencing target genes on ethylene sensitivity and abscission competence of the leaf and flower AZs; 3) To isolate and characterize promoters from AZ-specific genes to be used in functional analysis; 4) To generate stable transgenic tomato plants with selected genes silenced with RNAi, under the control of an AZ-specific promoter, for further characterization of their abscission phenotypes.

Background: Abscission, the separation of organs from the parent plant, results in postharvest quality loss in many ornamentals and other fresh produce. The process is initiated by changes in the auxin gradient across the AZ, and is triggered by ethylene. Although changes in gene expression have been correlated with the ethylene-mediated execution of abscission, there is almost no information on the initiation of the abscission process, as the AZ becomes sensitized to ethylene. The present project was focused on elucidating these early molecular regulatory events, in order to gain a better control of the abscission process for agricultural manipulations.

Major conclusions, solutions, achievements: Microarray analyses, using the Affymetrix Tomato GeneChip®, revealed changes in expression, occurring early in abscission, of many genes with possible regulatory functions. These included a range of auxin- and ethylene-related transcription factors (TFs), other TFs that are transiently induced just after flower removal, and a set of novel AZ-specific genes. We also identified four different defense-related genes, including: *Cysteine-type endopeptidase*, *α-DOX1*, *WIN2*, and *SDF2*, that are newly-associated with the late stage of the abscission process. This supports the activation of different defense responses and strategies at the late abscission stages, which may enable efficient protection of the exposed tissue toward different environmental stresses. To facilitate functional studies we implemented an efficient VIGS system in tomato, and isolated two abscission-specific promoters (pTAPG1 and pTAPG4) for gene silencing in stable transformation. Using the VIGS system we could demonstrate the importance of TAPGs in abscission of tomato leaf petioles, and evaluated the importance of more than 45 genes in abscission. Among them we identified few critical genes involved in leaf and flower abscission. These included: *PTRP-F1*, *PRP*, *TKN4*, *KNOTTED-like homeobox TF*, *KD1*, and *KNOX-like homeodomain protein* genes, the silencing of which caused a striking retardation of pedicel abscission, and *ERF1*, *ERF4*, *Clavata-like3 protein*, *Sucrose transporter protein*, and *IAA10* genes, the silencing of which delayed petiole abscission. The importance of *PRP* and *KD1* genes in abscission was confirmed also by antisense-silencing using pTAPG4. Experiments testing the effects of RNAi silencing of few other genes are still in progress. The analysis of the microarray results of flower and leaf AZs allowed us to establish a clear sequence of events occurring during acquisition of tissue sensitivity to ethylene, and to confirm our hypothesis that acquisition of ethylene sensitivity in the AZ is associated with altered expression of auxin-regulated genes in both AZs.

Implication, both scientific and agricultural: Our studies had provided new insights into the regulation of the abscission process, and shed light on the molecular mechanisms that drive the acquisition of abscission competence in the AZ. We pointed out some critical genes involved in regulation of abscission, and further expanded our knowledge of auxin-ethylene cross talk during the abscission process. This permits the development of novel techniques for manipulating abscission, and thereby improving the postharvest performance of ornamentals and other crops.

Achievements:

Significance of main scientific achievements or innovations

Abscission is a highly temporally and spatially regulated process in which various organs, including leaves, flowers and fruits are separated from the mother plant. This may result in postharvest quality loss in many ornamentals and other fresh produce. The abscission process is induced by ethylene, while the rate and degree of abscission depends upon the endogenous hormonal balance between auxin and ethylene levels in the tissue. Organ abscission is accompanied by a modified expression of various types of genes, including ethylene-inducible, auxin-responsive, pathogen-related (PR) genes, as well as genes encoding for cell-wall degrading enzymes. Despite long-lasting physiological, biochemical and molecular studies in various abscission systems, and evidences suggesting that changes in gene expression have been correlated with the ethylene-mediated execution of abscission, there is almost no information on the initiation of the abscission process, as the AZ becomes sensitized to ethylene. Also, the molecular mechanisms that drive the acquisition of abscission competence and its modulation by auxin gradients are still largely unknown. Our present project has provided some new insight into the basic understanding of these early molecular regulatory events operating in the abscission process of flowers and leaves, thereby partially filling this gap in knowledge. The significance of the main achievements gained in this project is briefly outlined as follows:

Our results from transcriptome analysis demonstrated global changes in gene expression in the tomato flower and leaf AZ tissues at an early stage following induction of the abscission process, when the AZ becomes sensitive to ethylene, as well as at later stages in the process during execution of pedicel or petiole abscission and the development of a defense layer. Accordingly, the results allowed the study of auxin-ethylene cross-talk at a wide scope of the abscission process with the aid of the newly identified abscission-related genes. Furthermore, to confirm that the observed changes in the transcriptome were result from auxin depletion but not from wounding (organ removal) or other non-related signals, the transcriptome analysis was performed using the AZ samples treated with exogenous IAA on the cut surface of the remaining pedicel or petiole tissues after organ removal. The results obtained further support our hypothesis that the observed changes in gene expression induced by flower removal or leaf deblading are due to auxin depletion. Based on the findings of this research obtained with tomato plants, we could separate the sequence of events which occur during tomato flower abscission into two phases: Early events (0 to 4 h after organ removal) that probably lead to acquisition of ethylene sensitivity and abscission competence, and late events (8 to 14 h after organ removal) when

processes leading to the execution of pedicel abscission and development of the defense layer occur. A similar trend was established for tomato leaves, but with a different time scale, including 1-2 days for the early events, and 3-4 days for the late events. We could also establish the following sequence of molecular events occurring in the AZ after organ removal: Genes showing early modified expression might be involved in mediating auxin regulation of ethylene sensitivity in the AZ. These include genes that are directly regulated by auxin, such as *Aux/IAA* genes, some of the TFs - the *knotted*, *Homeobox-Leu zipper* genes and *bHLH*; genes that are directly IAA-repressed such as *PK7*, *ERF1c*, *WRKY lld-1*, *Protein phosphatase*; other TF and/or post transcriptional regulators, such as *LRR-RLK*, *PK7*, *TPRP-F1*, *Phantastica*, and *Ovate*. As the AZ becomes sensitive to ethylene, the basic level of ethylene production together with its autocatalytic increase is mediated specifically in the AZ by specific expression of ethylene biosynthesis-related genes, such as *ACS*. This induction of ethylene levels leads in turn to activation of AZ-specific genes involved in the late stage of abscission and its execution after 4 h, including: TF genes or genes belonging to ethylene signal transduction or abscission regulators such as: *ETR4*, *CTR1*, *ERF1c*, *TAGL12*, *LRR-RLK*, and *PK7*; genes encoding cell wall modifying proteins; genes involved in the PR defense and development of the defense layer, such as *Cysteine-type endopeptidase*, *α -DOX1*, *WIN2*, and *SDF2*, that are newly-associated with the late stage of the abscission process. This supports the activation of different defense responses and strategies at the late abscission stages, which may enable efficient protection of the exposed tissue toward different environmental stresses.

Our research describes a unique attempt to basically understand the molecular changes occurring during acquisition of abscission competence in the AZ following auxin depletion, by introducing several novel ideas and developing or adopting powerful techniques, which show considerable promise. These include using of the Affymetrix Tomato GeneChip newly released when the project was initiated, development of an effective Virus-Induced Gene Silencing (VIGS) system as a fast tool for testing the role of the various abscission-related genes in the separation process, and isolation of two abscission-specific promoters (*pTAPG1* and *pTAPG4*) for silencing target genes in stable transformations.

Using the VIGS system we could demonstrate the importance of TAPGs in abscission of tomato leaf petioles, and evaluated the importance in abscission of more than previously identified 45 genes, that most of them were found in our earlier transcriptome analyses in tomato leaf and flower AZs to be induced prior to and during the cell separation process. Among them we identified a few critical genes involved in leaf and flower abscission, and gained a new insight into their possible roles in abscission as follows: 1) Silencing of a ribosome gene (*RPL2*) with an abscission specific promoter delayed leaf

abscission, suggesting new protein synthesis is required for onset of petiole abscission. 2) Antisense-silencing of a cell wall structural gene encoding a proline-rich protein (PRP), and of *KDI* gene encoding a novel KNOTTED1-LIKE HOMEODOMAIN protein that lacks homeodomain, using an abscission-specific promoter, delayed both pedicel and petiole abscission. These data suggest the importance of plant cell wall dynamic changes in the AZ during the abscission process, and that *KDI* plays a crucial role in regulating pedicel abscission through auxin-mediated response pathway. Perhaps *KDI* acts as a negative regulator that controls balance between auxin and ethylene.

The present study has established a powerful platform for further analysis of possible regulatory abscission-related genes involved in acquisition of ethylene sensitivity at the AZ, both in leaves and flowers. In parallel, we have initiated functional analysis of selected candidate genes, and some function in abscission was strongly suggested, based on the phenotypic consequences of modifying their expression. These genes are currently specifically inhibited using RNAi in stably transformed tomato plants, as regulated by an abscission-specific promoter. This functional analysis will enable us to further reveal the role of key regulators in the early events of the abscission process.

Taken together, this study with its rather innovative and unique findings, has shed more light on the process of organ abscission, and supplied new information on the involvement and the complex hormonal interactions of ethylene and auxin during the abscission process in the AZs of both flowers and leaves. As such, this study suggests possible useful means for controlling the abscission process, and has therefore important implications for science and agriculture.

Agricultural and/or economic impacts of the research findings

Organ abscission has an important impact on agriculture. Abscission can often result in a significant loss of quality and longevity in many ornamentals and other fresh produce. To overcome this problem, many crops receive postharvest chemical treatments to delay or prevent abscission, which have limited effects. Therefore, elucidation of the mechanisms involved in leaf and flower abscission may provide better tools and strategies for postharvest handling of cut flowers and other crops. The findings of this study shed light on the molecular mechanisms that drive the acquisition of abscission competence, thereby facilitating novel approaches to the control and manipulation of abscission in horticultural and agricultural crops, in order to improve their postharvest quality. This outcome is of great economic value to both countries.

Details of cooperation:

The cooperation between the two teams in Israel and USA was very synergistic. The results for the validation of the microarray analyses performed in Israel were sent to the American team and helped them with the selection of the most suitable genes for functional analysis by using the VIGS technology. The US team cloned the flower AZ-specific *TAPG4* promoter, and both teams used it for the functional analyses performed *via* stable transformation experiments in tomato.

The PI, Dr. Shimon Meir spent in the past a sabbatical year in UC Davis, in the lab of the Co-PI, Prof. Michael S. Reid. During this year they successfully isolated abscission-associated genes from *Mirabilis jalapa* (Meir et al., 2006), that served later on as the basis for the present study. Both research groups shared openly for long time useful information regarding handling and transportation of cut flowers, which is in great benefit for the flower industry in both countries. The two research teams from Israel and USA met in three international conferences: M. Reid, S. Meir and S. Philosoph-Hadas met in June 2007 in Madeira, Portugal; S. Meir, S. Philosoph-Hadas and A.J. Macnish met in August 2008 in Denmark; M. Reid, C.-Z. Jiang, S. Meir and S. Philosoph-Hadas met in April 2009 in Antalya, Turkey. Two researchers from Israel: the PI, S. Meir and the Co-PI, S. Philosoph-Hadas, visited UC-Davis in January 2008, and during their stay there they performed several experiments for functional analysis of several candidate genes using VIGS, to examine the consequences of their silencing to the abscission phenotype.

Apart of the visit of the Israeli researchers to UC-Davis and the joint meetings at scientific conferences, the two teams have a close contact *via* regular email communication, as well as common presentations in two conferences and publications resulting from this project, as detailed in the following list of publications.

List of Publications (only reviewed publications)

- Jiang, C-Z., Feng, L., Imsabai, W., Meir, S. and Reid, M.S. (2008). Silencing polygalacturonase expression inhibits tomato petiole abscission. *J. Exp. Bot.* 59: 973-979.
- Meir, S., Philosoph-Hadas, S., Sundaresan, S., Selvaraj, K.S.V., Burd, S., Ophir R., Kochanek, B., Reid, M.S., Jiang, C-Z. and Lers, A., (2010). Microarray analysis of the abscission-related transcriptome in tomato flower abscission zone in response to auxin depletion. *Plant Physiol.* 154: 1929-1956.
- Liang, Y-C, Imsabai, W., Wagstaff, C., Reid M.S. and Jiang. C-Z. (2010). A universal primer for isolation of fragments of a gene encoding phytoene desaturase for use in Virus-Induced Gene Silencing (VIGS) studies. *Acta Hort.* 877:1697-1703.
- Meir, S., Philosoph-Hadas, S., Sundaresan, S., Selvaraj, K.S.V., Burd, S., Ophir R., Kochanek, B., Reid, M.S., Jiang, C-Z. and Lers, A., (2011). Identification of defense-related genes newly-associated with tomato flower abscission. *Plant Signaling & Behavior* 6: 590-593.
- Jiang, C-Z, Chen J.C. and Reid. M.S. (2011). Virus-Induced Gene Silencing in Ornamental Plants. In: *Methods in Molecular Biology, RNAi and Plant Gene Function Analysis*, H. Kodama ed. Clifton, New Jersey, pp. 81-96.