**Final Scientific Report
Cover Page**

Date of Submission of the report:

Project Number: TIE 04-04

Project Title: Grafting as a strategy for disease and stress management in muskmelon production

Investigators

Principal Investigator (PI):

Roni Cohen _____

Co-Principal Investigator (Co-PI):

Kevin Crosby

Collaborating Investigators:

Beni Aloni _____

Menahem Edelstein _____

Nurit Katzir _____

Haim Nerson _____

John Jifon

Daniel Leskovar

Institutions

Agricultural Research Organization

Texas A&M University

Agricultural Research Organization

Agricultural Research Organization

Agricultural Research Organization

Agricultural Research Organization

Texas A&M University

Texas A&M University

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

Monosporascus cannonballus, vine decline, rootstock, graft compatibility

Abbreviations commonly used in the report, in alphabetical order:

Budget: IS: \$150,000

US: \$150,000

Total: \$300,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Final Scientific Report (page 2)

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) UMBI/I support acknowledged	0	5	3	8
Submitted, in review, in preparation	0	1	2	3
Invited review papers	0	0	1	1
Book chapters	0	0	0	0
Books	0	0	0	0
Master theses	0	0	0	0
Ph.D. theses	0	0	0	0
Abstracts	0	7	0	7
Not refereed (proceedings, reports, etc.)	6	0	0	6

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	0	0	0	0
Longer Visits (Sabbaticals)	0	0	0	0

Description Cooperation:

Joint planning of rootstock and pathogen selection for field experiments to maximize amount of useful data. We simultaneously examined the effects of different growth regulators on grafted plants' development and stress tolerance. We combined all data into reports and jointly reviewed manuscripts for publication. Collaboration on SEST 2006 conference presentations.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	0	0	0	0
Issued (allowed)	0	0	0	0
Licensed	0	0	0	0

Abstract. The overall objective of this research was to elucidate the horticultural, pathological, physiological and molecular factors impacting melon varieties (scion) grafted onto *M. cannonballus* resistant melon and squash rootstocks. Specific objectives were- to compare the performance of resistant melon germplasm (grafted and non-grafted) when exposed to *M. cannonballus* in the Lower Rio Grande valley and the Wintergarden, Texas, and in the Arava valley, Israel; to address inter-species relationships between a *Monosporascus* resistant melon rootstock and susceptible melon scions in terms of fruit-set, fruit quality and yield; to study the factors which determine the compatibility between the rootstock and the scion in melon; to compare the responses of graft unions of differing compatibilities under disease stress, high temperatures, deficit irrigation, and salinity stress; and to investigate the effect of rootstock on stress related gene expression in the scion. Some revisions were- to include watermelon in the Texas investigations since it is much more economically important to the state, and also to evaluate additional vine decline pathogens *Didymella bryoniae* and *Macrophomina phaseolina*. Current strategies for managing vine decline rely heavily on soil fumigation with methyl bromide, but restrictions on its use have increased the need for alternative management strategies. Grafting of commercial melon varieties onto resistant rootstocks with vigorous root systems is an alternative to methyl bromide for *Monosporascus* root rot/vine decline (MRR/VD) management in melon production. Extensive selection and breeding has already produced potential melon rootstock lines with vigorous root systems and disease resistance. Melons can also be grafted onto *Cucurbita* spp., providing non-specific but efficient protection from a wide range of soil-borne diseases and against some abiotic stresses, but compatibility between the scion and the rootstock can be problematic. During the first year experiments to evaluate resistance to the vine decline pathogens *Monosporascus cannonballus*, *Didymella bryoniae*, and *Macrophomina phaseolina* in melon and squash rootstocks proved the efficacy of these grafted plants in improving yield and quality. Sugars and fruit size were better in grafted versus non-grafted plants in both Texas and Israel. Two melons (1207 and 124104) and one pumpkin, Tetsukabuto, were identified as the best candidate rootstocks in Texas field trials, while in Israel, the pumpkin rootstock RS59 performed best. Additionally, three hybrid melon rootstocks demonstrated excellent resistance to both *M. cannonballus* and *D. bryoniae* in inoculated tests, suggesting that further screening for fruit quality and yield should be conducted. Experiments with ABA in Uvalde demonstrated a significant increase in drought stress tolerance and concurrent reduction in transplant shock due to reduced transpiration for ‘Caravelle’ plants. In Israel, auxin was implicated in reducing root development and contributing to increased hydrogen peroxide, which may explain incompatibility reactions with some squash rootstocks. However, trellised plants responded favorably to auxin (NAA) application at the time of fruit development. Gene expression analyses in Israel identified several cDNAs which may code for phloem related proteins, cyclins or other factors which impact the graft compatibility. Manipulation of these genes by transformation or traditional breeding may lead to improved rootstock cultivars. Commercial applications of the new melon rootstocks as well as the ABA and TIBA growth regulators have potential to improve the success of grafted melons in both Israel and Texas. The disease resistance, fruit quality and yield data generated by the field trials will help producers in both locations to decide what rootstock/scion combinations will be best.

Scientific Achievements and Innovations:

Physiology

We demonstrated that rootstocks with vigorous root systems are able to confer tolerance to soil-borne disease infection by rapid regeneration of new roots thus allowing infected plants to meet the increased water/nutrient demands during fruit maturation. Melon lines 1207 and 124104 performed well in both Texas locations, and had significantly larger root areas when analyzed by Rhizo Pro computer software. Application of ABA demonstrated some potential to reduce drought stress, which impacts vine decline. In Israel, auxin was demonstrated to have a negative impact on root development and blocking it with TIBA was demonstrated as a potential means to improve rootstock-scion compatibility. NAA application lead to increased hydrogen peroxide, which damages the root cells. However, in trellised plants, NAA demonstrated a positive impact on root mass when applied around fruit set.

Pathology

Both field and greenhouse experiments with inoculation of melon and squash rootstocks demonstrated the functionality of resistance to vine decline, against three pathogens- *M. cannonballus*, *D. bryoniae* and *M. phaseolina*. Resistance to root rot was demonstrated under inoculation with high colony forming units in sand culture in melon lines 1207 and 124104, while field trials demonstrated potential for high quality fruit production. Superior early root vigor was also demonstrated in these two lines, and likely impacts resistance to vine decline and capacity to regenerate fine roots. Due to the potential for total crop loss, resistance to these pathogens must be deployed at locations in both Texas and Israel. The melon lines 1207 and 124104 may help overcome fruit quality and incompatibility issues. The Texas squash rootstocks J1 and L1 demonstrated excellent potential for disease resistance, but demonstrated very low fruit set and yield compared to the melon rootstocks at Weslaco. In contrast, several squash rootstocks, including RS 59, Tetsukabuto and J1 provided acceptable yields and quality when grafted with hybrid watermelon. Squash rootstock TX148 resistance to *Macrophomina* in Israel field trials resulted in much greater yields of marketable melons, despite susceptible scions.

Gene Expression

Differential gene expression was investigated by suppression subtractive hybridization between different cDNA pools, grafted scions vs. rootstock and grafted vs. non-grafted scions. The Macroarray procedure was used to identify 70 differentially expressed clones, 20 of which were investigated. Expression patterns of these genes measured by real time PCR revealed several genes induced more highly in compatible graft combinations. Several shared similarity with genes coding phloem proteins and another with a gene for cyclin D 3.1, affecting leaf development. These cloned gene sequences are candidates for further investigations into specific regulatory roles, which positively or negatively impact graft compatibility. They could be utilized for genetic transformation to evaluate the impact of up or down regulation on grafted plant performance. Additionally, they may be utilized as molecular markers in a rootstock breeding program to develop improved lines.

Agricultural and/or economic impacts:

Texas and Israel are important producers of Cucurbit (melons, watermelons, squashes etc) crops. Texas has about 80,000 acres planted annually for a combined value of nearly \$195 million (Anciso and Smith, 2005). Increasing limitations on new land for crop rotation has lead to a buildup of soil-borne pathogens and has increased the need for alternative disease management strategies such as grafting. It is anticipated that as the cost of planting grafted seedlings declines, this technology will contribute towards the economic sustainability of the vegetable industry as well as replace the use of chemical treatments such as soil fumigation with environmentally unsafe compounds for soil-borne disease management. Several melon and squash rootstocks investigated in this project proved suitable for commercial application, due to excellent vine decline resistance. In Israel, extensive application of grafted melons and watermelons already exists. Identification of the best rootstock scion combinations as well as growth regulators to enhance compatibility by this research will improve overall productivity and resistance to vine decline. Identification

of the molecular mechanisms of graft incompatibility may allow genetic transformation and traditional breeding to eliminate detrimental genes or select for beneficial ones. Candidate genes have been elucidated by the investigations in Dr. Katzir's lab.

Details of Cooperation:

Cooperation between investigators as well as between investigators and major commercial melon/watermelon growers (A&W Produce, Weslaco; Noel Borders) was critical in achieving our objectives and elucidating the mechanisms of disease tolerance in grafted plants. We focused on complementary research with the potential to generate future collaboration, rather than repeating experiments in each country. In both countries, the field trials provided contrasting locations (infested and non-infested) for evaluating the benefits and mechanisms of disease protection by grafting. As a result three distinct pathogens were evaluated. Different growth regulators were evaluated by Israeli and Texas investigators to generate more expansive data for publication. Melon rootstocks were a primary focus in the Texas experiments at the suggestion of the Israeli investigators, who already had extensive experience with squash. Genes derived from SSHR are now candidates for inclusion in melon genome maps at both institutions.

Publications:

Peer-Reviewed:

1. Aloni, B., Karni, L., Deventurero G., Levin, Z., Cohen, R., Katzir, N., Lotan-Pompan, M., Edelstein, M., Aktas, H., Turhan, E., Joel D.M and Horev, C. 2008. Possible mechanisms for graft Incompatibility Between Melon Scions and Pumpkin Rootstocks *Acta Horticulturae* 782 : 23-33
2. Cohen, R, Burger, Y., Horev, C., Koren, A. and Edelstein, M. 2007. Introducing grafted cucurbits to modern agriculture: The Israeli experience. *Plant Dis.* 91:916-923. **Feature article**
3. Edelstein, M., Ben-Hur, M. and Plaut, Z. 2008. Response of grafted and non-grafted melon plants to effluent and boron under greenhouse conditions. *Acta Hort.* 782: 325-328
4. Crosby, K.M., J.L. Jifon, and D.I. Leskovar. 2008. Genetic Improvement of Early Root Vigor in Melon (*Cucumis melo* L.) to Enhance Stand Establishment. *Acta Hort.* 782:273-278.
5. Goreta, S., D.I. Leskovar, and J.L. Jifon. 2007. Gas Exchange, Water Status, and Growth of Pepper Seedlings Exposed to Transient Water Deficit Stress are Differentially Altered by Antitranspirants. *J. Amer. Soc. Hort. Sci.* 132:603-610.
6. Jifon, J.L., K.M. Crosby, D.I. Leskovar and M. Miller. 2008. Possible Physiological Mechanisms for Resistance to Vine Decline Diseases in Grafted Watermelons. *Acta Hort.* 782:329-334.
7. Jifon JL, KM Crosby, ME Miller and DI Leskovar. 2006. Physiological Characteristics of Grafted Muskmelon Grown in *Monosporascus cannonballus*-infested Soil in South Texas. Pages 23-30 in: *Proceedings of Cucurbitaceae 2006*. G.J. Holmes, ed. University Press, North Carolina.
8. Leskovar, D.I. S.Goreta, Jifon JL, S. Agehara, T. Shinohara, and D. Moore. 2007. ABA to Enhance Water Stress Tolerance of Vegetable Transplants. *Proceedings of the 4th International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops, "Translating seed and seedling physiology into technology"*, San Antonio, Texas, USA, December 3-6, 2006 under the auspices of the International Society for Horticultural Science, ISHS. *Acta Hort* – In Press.

Articles in preparation

9. Lotan-Pompan, M., Portnoy, V., Edelstein, M., Cohen, R., Joel, D., Aloni, B. Karni, L., Deventurero, h. Aktas, Z. Levin, Y. Kapulnik, y. and Katzir, N. 2008. Identification of gene expression at the scion-rootstock

interface of grafting combinations between melon scion and pumpkin rootstocks

10. Aloni, B., Karni, L., Deventurero, G., Levin, Z., Cohen, R., Katzir, N., Lotan-Pompan, M., Edelstein, M., Aktas, H., Joel, D., Horev, C. and Kapulnik, Y. 2008. Physiological and biochemical changes occurring in scion-rootstock interface in grafting combinations between melon scion and pumpkin rootstocks.

Book chapter

1. Edelstein, M., Cohen, R. and Ben-Hur, M. (2008). Using grafted vegetables to increase tolerance to salt, toxic elements, and soil-borne diseases In: Agriculture in arid and semiarid zones: Soil, water, and environmental aspects, (M. Ben- ur, ed.), Research Signpost. (accepted).

Popular-Press articles:

1. Seedquest® News Section; July 7, 2005. "Texas Agricultural Experiment Station Scientists believe that grafting may help battle melon vine decline"
2. Border Business, Friday July 8th 2005: Grafting helps fight melon vine decline" by Rod Santa Ana III.
3. Valley Morning Star, Sunday July 10, 2005 "Grafting may help battle melon vine decline" by Rod Santa Ana III.
4. El Valle, Miercoles 13 julio de 2005 "Injertos a melons enfermos" by Rod Santa Ana III.
5. Mid-Valley Town Crier, Wednesday, July 13, 2005 "Grafting may help battle melon vine decline" by Rod Santa Ana III.
6. The Vegetable Growers News. Dec. 2006. Grafting may help battle melon vine decline. Article by Dick Lehnert.

Appendix

Table of Contents

	<u>Page</u>
Possible mechanisms for graft incompatibility between melon scions and pumpkin rootstocks.....	2-12
Introducing grafted cucurbits to modern agriculture- The Israeli experience.....	13-20
Response of grafted and non-grafted melon plants to effluent and boron under greenhouse conditions.....	21-24
Genetic Improvement of Early Root Vigor in Melon (<i>Cucumis melo</i> L.) to Enhance Stand Establishment.....	25-30
Gas exchange, water status, and growth of pepper seedlings exposed to transient water deficit stress are differentially altered by antitranspirants.....	31-38
Possible Physiological Mechanisms for Resistance to Vine Decline Diseases in Grafted Watermelons	39-44
Physiological characteristics of grafted muskmelon grown in <i>Monosporascus cannonballus</i> infested soil in South Texas.....	45-53
ABA to enhance water stress tolerance of vegetable transplants.....	54-65
Physiological and biochemical changes occurring in scion-rootstock interface in grafting combinations between melon scion and <i>Cucurbita</i> rootstocks.....	66-87
Popular Press Articles.....	88-91
Unpublished Data.....	92-96

Possible Mechanisms for Graft Incompatibility between Melon Scions and Pumpkin Rootstocks

Beny Aloni¹, Leah Karni¹, Gila Deventurero¹, Ziv Levin¹, Ron Cohen², Nurit Katzir², Maya Lotan-Pompan², Menahem Edelstein², Hakan Aktas³, Ece Turhan⁴, Daniel M. Joel⁵, Carmella Horev⁵ and Yoram Kapulnik

¹ Dept. of Vegetable Research, Institute of Plant Science, A.R.O. the Volcani Center, B.O.B. 6, Bet-Dagan, 50250, Israel

² Dept. of Vegetable Research, Institute of Plant Science, A.R.O. the Volcani Center, Neve Ya'ar, Israel

³ Harran University, Faculty of Agriculture, Department of Horticulture, 63040 Sanliurfa, Turkey

⁴ Canakkale, Onsekiz Mart University, Boyramic Vocational College, 17700 Boyramic/Canakkale, Turkey

⁵ Division of Weed Research, A.R.O. the Volcani Center, Neve-Ya'ar, Israel

⁶ Dept. of Agronomy and Natural Resources, A.R.O. the Volcani Center, B.O.B. 6, Bet-Dagan, 50250, Israel

Keywords: grafting, melon, pumpkin, rootstock, compatibility, auxin, ethylene, oxidative stress, high temperature

Abstract

The successful application of the grafting technique depends on the identification of stress and pathogen-resistant rootstock (RS), and on the compatibility of the grafted union in terms of fast formation of the vascular connections between the rootstock and the scion and rapid resumption of root and shoot growth. A series of studies, using compatible and incompatible *Cucurbita* rootstocks, grafted with melon (*Cucumis melo* L.) scion ('Arava'), were conducted in order to identify metabolic and physiological factors which may be associated with the level of grafting compatibility. In order to increase the differential compatibilities, the grafted seedlings were exposed to either control (22/28°C) or high (28/35°C) temperature conditions starting at 7 days after grafting. Grafting compatibility was dependent on temperature conditions. Twenty days from grafting the rootstock part collapsed only in the non-compatible grafted seedlings under high temperatures but not under normal temperatures. Water uptake and sugar distribution between plant canopy and root measured 14 days under these conditions, and before incompatible transplants collapsed, were not correlated with the differential compatibility. At that stage histo-chemical staining showed that reactive oxygen species (ROS) generation was enhanced under high temperatures in the grafting zone in both compatible and incompatible graft combinations. In this paper we describe a mechanism which may be responsible for grafting incompatibility between *Cucurbita* RS and melon scion. We suggest that upon formation of the RS-scion continuum, the hormonal balance, especially of auxin and ethylene, required for proper root development, is impaired in incompatible grafting union. This impairment is initiated by the auxin, transported from the scion to the root, which in incompatible RS, triggers ethylene production, ROS generation and root growth inhibition and degeneration. In compatible RS, a variety of mechanisms may operate to either reduce auxin activity in the RS and/or reduce ROS production and activity.

INTRODUCTION

Muskmelon (*Cucumis melo*) production represents a significant component of the vegetable industry throughout the world. However, productivity of this warm season crop does not reach its full potential mostly due to drought, extreme temperatures, and to devastating soil-borne diseases. Methyl bromide has been the primary pre-plant soil

fumigant for controlling the causal agent. However, this chemical is banned due to its high environmental toxicity. Therefore, there is an urgent need to develop management strategies aimed at maintaining production and viability of the melon industry. Grafting melons onto vigorous, disease-resistant rootstocks is an alternative to chemical control. Besides providing resistance against soil-borne diseases, grafting may provide tolerance to few abiotic stresses, such as heat stress (Rivero et al., 2003), low root temperature (Bulder et al., 1990) and boron and saline toxicity (Edelstein et al., 2005). Grafting is a well known technique in Korea, Japan and Mediterranean countries. However, problems related to rootstock-scion incompatibility, poor grafting techniques, physiological disorders leading to low plant survival, poor stand establishment, low yield/quality, and high production costs have limited their use elsewhere. Grafting technology has been documented in few reviews in which the technical and horticultural aspects of grafting were described (Lee, 1994, 2003; Lee and Oda, 2003). However, the mechanisms involved in determination of grafting compatibility/incompatibility have not been elucidated yet.

The successful application of the grafting technique depends on the identification of stress and pathogen-resistant rootstocks, and on the compatibility of the graft union in terms of fast formation of the vascular connection between the root stock and the scion and fast renewal of root and canopy growth.

Melons can be grafted onto *Cucurbita* spp. as well as *Cucumis melo* rootstocks. Each rootstock spp has its beneficial characteristics and disadvantages (Cohen et al., 2002; Edelstein et al., 1999, 2004). *Cucurbita* rootstocks provide non-specific but efficient protection from a wide range of soil-borne diseases and against some abiotic stresses. However, compatibility between the scion and rootstock species varies. Field observations indicate that grafting compatibility depends on the season and climate which prevails at planting time. For example, in Israel, melons grafted on *Cucurbita* rootstocks are more successful when planted in the spring than if planted in the autumn.

Edelstein et al. (2004) have selected interspecific *Cucurbita* rootstocks with differential compatibilities when grafted with the melon 'Arava'. The present paper summarizes experiments that we have conducted in order to learn about possible mechanisms which determine the differential compatibility of *Cucurbita* rootstocks and *Cucumis melo*, 'Arava' scions. Collectively, it was found that the disruption of rootstock-scion connections in incompatible grafting is secondary to the proposed main cause of incompatibility that is the occurrence of hormonal imbalance, primarily, of auxin and ethylene in the root system following the establishment of the grafting connections.

MATERIALS AND METHODS

Two pumpkin (*C. moschata* × *C. moschata*) genotypes (RS59, RS62) and one melon cultivar (*Cucumis melo*, 'Arava') were used as rootstocks. Melon ('Arava') was used as scion. Based on data from Edelstein et al. (2004), RS62 and RS59 are incompatible and compatible with the melon scion ('Arava'), respectively. Whip grafting was carried out by Hishtil Nurseries Co. (Ashkelon, Israel). After maintaining the grafted and non-grafted seedlings in the nursery for 7 days, the seedlings were transferred to the Volcani Center Phytotron and were divided between two growth chambers with: (A) normal temperature conditions (22/26°C, night/day), (B) high temperature (28/35°C).

Measurements of water uptake by the grafted seedlings were done by two methods; potometry (Din, 2003; Kari and Lars, 2004) and by following uptake of water soluble dye (safranin O).

Histochemical staining for the presence and location of superoxide, peroxidase and H₂O₂ in the grafting zone was carried out according to Barceló (1998). In short, stem segments of ca. 4 cm, 2 cm on both sides of the grafting zone, were cut off. Cross-sections through the RS stem, the grafting area and the scion stem were made and sections of 2 mm were prepared and were incubated in a reaction solution which included nitroblue tetrazolium (NBT). At the end of the reaction the stained sections were observed and photographed.

Extraction and Enzymatic Assays

The procedure of extraction and enzymatic assay of peroxidase from the grafting zone and the root systems were carried out according to Andrews et al. (2000). Super oxide dimutase (SOD) was extracted and assayed according to McCord and Fridovich (1969). H_2O_2 was determined according to Ngo and Lenhoff (1980). Peroxidase (POX) isoenzymes in the grafting zone stem section and in the roots were separated in non-denaturing polyacrylamide gels according to Andrews et al. (2000).

Growth Regulator Treatments

Growth regulators (naphthalene acetic acid: NAA, TIBA: 2,3,5-triiodobenzoic acid, Ethrel, ethylene inhibitors; AVG: α -(2-aminoethoxyvinyl)-glycine, STS: silver thiosulfate) were applied to nursery-grown transplants (14 days after grafting) by various methods: (A) NAA at various concentrations was applied as single foliar spray given to the transplants following planting them in 2-L pots filled with perlite growth medium, (B) NAA, Ethrel and ethylene inhibitors were also applied as root treatment in hydroponics or (C) by floating the transplanting trays over nutrient solutions containing the growth regulators, in 20 liter-containers. (D) The auxin transport inhibitor; TIBA, was applied at different concentrations to the stem as lanolin paste. The transplants were sampled and examined after various period of incubation up to 20 days of treatment.

RESULTS AND DISCUSSION

Exposure of the grafted transplants to high temperature regime (26/32°C), after grafting establishment, resulted in substantial inhibition of root and shoot development of the incompatible grafted 'Arava'/RS62 plants as compared to the development of 'Arava'/RS59, 'Arava'/'Arava' and the non-grafted 'Arava' plants (Table 1). After 34 days from grafting, 30% of the seedlings of the 'Arava'/RS62 transplants, collapsed, but not seedlings of 'Arava'/RS59, 'Arava'/'Arava' or of non-grafted melon transplants of 'Arava' (not shown). Such collapse was not observed under control temperatures (22°C/28°C). Morphologically, it appeared that transplant collapse was initiated in the rootstock part while the scion part remained intact (Figs. 1 and 2). Our goal was to investigate possible mechanisms by which this grafting incompatibility is caused. Measurements of water uptake and stem hydraulic conductance, before grafting collapse had been observed, did not reveal a barrier to water passage through the stem in either compatible or incompatible grafting combinations (data not shown). Safranin 0 uptake indicated that the grafted pumpkin rootstocks transported more safranin 0 from the root to the shoot than the grafted melon rootstocks, regardless to their level of compatibility (not shown), indicating that water uptake is not limited in the grafting unions.

We have therefore decided to investigate the possibility that differential compatibility between pumpkin rootstocks and melon scions is a result of perturbation of hormonal balance. Since it is well established that the auxin, indoleacetic acid (IAA), is translocated from the shoot to the root, we have suspected that this hormone may trigger rootstock decay in incompatible grafting. IAA is known to have a central role in root development and function (Tanimoto, 2005), but when applied exogenously at relatively high concentrations (10^{-7} – 10^{-5} M), it has been reported to inhibit root development (Mulkey et al., 1982). We have tested the effects of exogenous auxin application on roots by foliar sprays with the synthetic auxin, naphthalene acetic acid (NAA), at relatively higher concentrations (10^{-4} M) than previously reported. As expected, the growth of roots of non-grafted pumpkin and of melon seedlings was inhibited and the root system of the incompatible RS62 was more affected than the compatible RS59 or melon RS 'Arava'. We have also applied NAA directly to the root in hydroponically-grown grafted transplants. The results show that at 14 days after grafting, NAA application caused root decay in the incompatible 'Arava'/RS62 and was much less harmful to 'Arava'/RS59, 'Arava'/'Arava' and non-grafted 'Arava' transplants (Table 2). The involvement of auxin in the incompatibility mechanism emerged from three additional observations: (A) endogenous IAA analysis revealed that the roots and stem (below the grafting zone) of

‘Arava’/RS62 contained high IAA concentrations than these tissues on the other grafting combinations (Fig. 3); (B) application of the auxin transport inhibitor, TIBA, in a lanolin paste on the stem of the grafted transplants, negated root degradation in the incompatible ‘Arava’/RS62, whereas it had only slight effect on the compatible grafting combinations (Fig. 4); (C) In partially girdled, incompatible ‘Arava’/RS62 combination, root and shoot development was normal (Fig. 5). Thus, taken together, these results support our notion that auxin, produced in the scion (‘Arava’), is translocated downwards to the root after grafting connection is established, and when reaching a threshold concentration, triggers degradative processes, causing root decay. We suggest that the incompatible pumpkin rootstock, RS62, is either more sensitive or accumulates more auxin than the compatible rootstocks, RS59 and ‘Arava’; therefore, root decay in this genotype is highly incompatible with ‘Arava’ melon scion.

One of the possible mechanisms that triggers degradative processes in the rootstock might be oxidative stress activities which are known to induce cell death symptoms in plant tissue (Yamamoto et al., 2003; Tamas et al., 2006). Reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) are produced under normal conditions (Mittler, 2002; Foyer and Noctor, 2003; Bartoli et al., 2004). However, under abiotic and biotic stresses they are produced in elevated concentrations and become harmful, resulting in lipid peroxidation, protein oxidation and damage to nucleic acids. We have therefore investigated the effect of NAA on three important oxidative stress activities; hydrogen peroxide (H_2O_2) as an ROS agent, and SOD and peroxidase as antioxidant enzymes (Schoner and Krause, 1990).

As shown in Table 2, H_2O_2 concentration in the root increased with increasing NAA concentration in the root medium, most notably in the incompatible grafted transplants of ‘Arava’/RS62. The compatible ‘Arava’/RS59 and ‘Arava’/‘Arava’ accumulated significantly less H_2O_2 in their roots. On the other hand, the activities of peroxidase and SOD in the root system of transplants of ‘Arava’/RS59 and ‘Arava’/‘Arava’ were increased by NAA treatment and to higher levels than in the root of ‘Arava’/RS62 (Table 2). Root peroxidase isozyme patterns were greatly altered by NAA treatment. While in melon roots, either grafted or non-grafted, peroxidase isozymic activity increased with NAA treatment, but its pattern was not changed. In pumpkin roots there was a significant modification of isozymic pattern (Fig. 7). Such modifications may point to altered compartmentation of the enzyme within the cells. However, since we could not detect differences in peroxidase isozymic pattern between pumpkin rootstocks, RS62 and RS59, we still don’t know the significance of these changes. Overall, these results support our assumption that incompatibility may result from NAA-induced oxidative stress in the rootstock, which may lead to enhanced H_2O_2 production. In compatible grafting, anti-oxidative mechanism(s) may be activated, therefore reducing H_2O_2 production in the root and enabling its growth.

Several reports suggest that root inhibition by high auxin concentrations may be caused by auxin-induced ethylene (Mulkey et al., 1982; Rahman et al., 2001). We have explored this possibility by measuring the effect of exogenously applied NAA on ethylene production by the roots of the grafted transplants. Fig. 5 shows that ethylene production in the root is increased several fold following 3 days of maintenance in $10^{-6}M$ NAA in hydroponics. There were no differences in root ethylene production between the different grafted transplants. However, roots of the incompatible ‘Arava’/RS62 treated with NAA, produced more ethylene than the rest of the compatible graft combinations (Fig. 6). We therefore propose that NAA may induce rootstock growth inhibition and subsequently, degradation in incompatible grafting combinations by enhancing ethylene production in the root. Ethylene may trigger H_2O_2 and other ROS production which may lead to root degradation and to growth inhibition of these grafted transplants.

On the basis of these findings we have developed a method by which pumpkin rootstocks are screened for their compatibility. This method is based on root sensitivity to treatments with high auxin or high ethylene concentrations.

In addition, an attempt is being made to identify genes and gene products for

molecular screening of compatible rootstocks. Such molecular markers might be related to root response to high auxin or high ethylene concentration. They may also be used in marker-assisted breeding of compatible, stress-resistant rootstocks.

Literature Cited

- Andrews, J., Malone, M., Thompson, D.S., Ho, L.C. and Burton, K.S. 2000. Peroxidase isozymes in the skin of maturing tomato fruit. *Plant Cell & Environ.* 23:415-422.
- Bartoli, C.G., Gomez, F., Martinez, D.E. and Guiamet, J.J. 2004. Mitochondria are the main target for oxidative damage in leaves of wheat (*Triticum aestivum* L.) *J. Exp. Bot.* 55:1663-1669.
- Barcelo, A.R. 1998. The generation of H₂O₂ in the xylem of *Zinnia elegans* is mediated by an NADPH-oxidase-like enzyme. *Planta.* 207:207-216.
- Bulder, H.A.M., van Hasselt, P.R., Kuiper, P.J.C., Speek, E.J. and den Nijs, A.P.M. 1990. The effect of low root temperature on growth and lipid composition of low temperature tolerant rootstock genotypes of cucumber. *J. Plant Physiol.* 138:661-666.
- Cohen, R., Horev, C., Burger, Y., Shreiber, S., Hershenhorn, J., Katan, J. and Edelstein, M. 2002. Horticultural and pathological aspects of Fusarium wilt management using grafted melons. *Hort. Sci.* 37:1069-1073.
- Din, Y.Y. 2003. Developing a better understanding of the relationship between transpiration and water uptake in plants. *Journal of Science Education and Technology*, 12:13-19.
- Edelstein, M., Burger, Y., Horev, C., Porat, A., Meir, A. and Cohen R. 2004. Assessing the effect of genetic and anatomic variation of Cucurbita rootstocks on vigor, survival and yield of grafted melons. *J. Hort. Sci. Biotech.* 79:370-374.
- Edelstien, M., Cohen, R., Shreiber, S., Pivonia, S. and Shtienberg, D. 1999. Integrated management of sudden wilt in melons, caused by *Monosporascus cannonballus*, using grafting and reduced rates of methyl bromide. *Plant Dis.* 83:1142-1145.
- Edelstein, M., Ben-Hur, M., Cohen, R., Burger, Y. and Ravina, I. 2005. Boron and salinity effects on grafted and non-grafted melon plants. *Plant and Soil* 269:273-284.
- Foyer, C.H. and Noctor, G. 2003. Redox sensing and signaling associated in plants: a re-evaluation of the concept of oxidative stress response in leaves. *J. Exp. Bot.* 53:1249-1254.
- Lee, J.M. 2003. Advances in vegetable grafting. *Chronica Hort.* 43:13-19.
- Lee, J.M. 1994. Cultivation of grafted vegetables. I. Current status, grafting methods, and benefits. *Hort. Sci.* 29:235-239.
- Lee, J.M. and Oda, M. 2003. Grafting of herbaceous vegetable and ornamental crops. *Hort. Rev.* 28:61-124.
- Kari, L.H. and Lars, S. 2004. Water uptake through sweet cherry (*Prunus avium* L.) fruit pedicels: Influence of fruit surface water status and intact fruit skin. *Acta Agric. Scan.* 54:91-96.
- McCord, J.M. and Fridovich, I. 1969. Superoxide dismutase. *J. Biol. Chem.* 244:6049-6055.
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7:405-410.
- Mulkey, T.J., Kuzmanoff, K.M. and Evans, M.L. 1982. Promotion of growth and shift in the auxin dose/response relationship in maize roots treated with the ethylene biosynthesis inhibitors aminoethoxyvinylglycine and cobalt. *Plant Sci. Let.* 25:43-48.
- NgO, T.T. and Lenhoff, H.M. 1980. A sensitive and versatile chromogenic assay for peroxidase and peroxidase-coupled reactions. *Anal. Biochem.* 105:389-397.
- Rahman, A., Amakawa, T., Goto, N. and Tsurumi, S. 2001. Auxin is a positive regulator of ethylene-mediated response in the growth of *Arabidopsis* roots. *Plant Cell Physiol.* 42:301-307.
- Rivero, R.M., Ruis, J.M., Sanchez, E. and Romero, L. 2003. Does grafting provide tomato plants an advantage against H₂O₂ production under conditions of thermal shock. *Physiol. Plant.* 117:44-50.

- Schoner, S. and Krause, G.H. 1990. Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. *Planta* 180:383-389.
- Tamas, L., Huttova, J., Mistrik, I., Simonovicova, M. and Siroka, B. 2006. Aluminium-induced drought and oxidative stress in barley roots. *J. Plant Physiol.* 163:781-784.
- Tanimoto, E. 2005. Regulation of root growth by plant hormones-roles for auxin and gibberellin. *Critic. Rev. Plant Sci.* 24:249-265.
- Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S. and Matsumoto, H. 2003. Oxidative stress triggered by aluminum in plant roots. *Plant and Soil.* 255:239-243.

Tables

Table 1. The effect of temperature treatments applied for 7 days, starting from 7 days after grafting on the development of melon (Ar, 'Arava') grafted on either melon or pumpkin rootstocks. Normal temperatures were: 22/26°C and high temperatures were: 28/35°C (night/day). Data are means (n = 10) ± S.E.

Temperature	Scion/Rootstock	Fresh weight (g)			
		Root		Shoot	
		Days after grafting			
		14	32	14	32
Normal 22/26°C	Ar (non grafted)	1.4 ± 0.1	11.9 ± 1.1	4.1 ± 0.3	50.5 ± 4.7
	Ar/Ar	1.8 ± 0.1	22.6 ± 1.7	4.1 ± 0.1	77.3 ± 6.7
	Ar/RS59	1.1 ± 0.1	13.8 ± 2.5	3.7 ± 0.1	74.8 ± 10.3
	Ar/RS62	1.0 ± 0.1	6.8 ± 2.4	3.9 ± 0.2	39.9 ± 2.1
High 28/35°C	Ar (non grafted)	2.5 ± 0.2	9.9 ± 1.1	6.1 ± 0.4	70.2 ± 5.6
	Ar/Ar	2.7 ± 0.3	9.9 ± 0.1	6.1 ± 0.3	78.9 ± 1.3
	Ar/RS59	2.0 ± 0.1	11.8 ± 1.0	5.8 ± 0.4	78.3 ± 3.8
	Ar/RS62	1.5 ± 0.2	4.70 ± 1.4	2.5 ± 0.1	12.1 ± 0.1

Table 2. The effect of NAA, applied for 7 days to the root medium, on the development of the grafted transplants and on H₂O₂ concentration, POX and SOD activities in the transplants' roots. Data are means (n = 5) ± S.E.

Scion/Rootstock	NAA (M)	Shoot fresh weight (g)	Root fresh weight (g)	H ₂ O ₂ (nmole/gFW)	POX activity (Unit/gFW)	SOD (Unit/gFW)
Ar (non-grafted)	0	190 ± 4	31 ± 4	19 ± 3	123 ± 3	1819 ± 103
	10 ⁻⁷	167 ± 7	32 ± 4	37 ± 6	172 ± 6	1104 ± 100
	10 ⁻⁶	144 ± 6	21 ± 2	65 ± 6	281 ± 4	6841 ± 586
	10 ⁻⁵	98 ± 2	18 ± 2	71 ± 3	340 ± 5	5878 ± 192
Ar/RSAr	0	125 ± 11	30 ± 2	16 ± 2	127 ± 5	1610 ± 152
	10 ⁻⁷	121 ± 9	33 ± 4	32 ± 2	178 ± 5	1226 ± 130
	10 ⁻⁶	105 ± 12	19 ± 1	70 ± 8	276 ± 5	6512 ± 655
	10 ⁻⁵	90 ± 7	20 ± 3	69 ± 7	352 ± 6	4992 ± 141
Ar/RS59	0	143 ± 8	15 ± 2	44 ± 5	122 ± 4	1471 ± 119
	10 ⁻⁷	135 ± 12	21 ± 4	30 ± 4	195 ± 4	2162 ± 268
	10 ⁻⁶	124 ± 10	15 ± 1	96 ± 9	203 ± 4	2365 ± 279
	10 ⁻⁵	72 ± 4	15 ± 1	65 ± 4	325 ± 1	3892 ± 126
Ar/RS62	0	23 ± 2	4 ± 0.4	46 ± 3	76 ± 1	900 ± 79
	10 ⁻⁷	36 ± 4	5 ± 0.3	35 ± 4	167 ± 3	1615 ± 188
	10 ⁻⁶	11 ± 1	1 ± 0.2	132 ± 8	172 ± 3	1608 ± 32
	10 ⁻⁵	6 ± 0.4	0.5 ± 0.03	194 ± 6	183 ± 3	2301 ± 224

Table 3. The effect of TIBA, applied in a lanolin paste at various concentrations, on the grafting zone of the grafted transplants, 10 days from grafting. Growth continued for 30 days following the application of the TIBA. Data are means (n = 15).

TIBA (ppm)	Arava/RS59		Arava/RS62	
	Shoot	Root Fresh weight (g)	Shoot	Root
Control	37.7	9	6.1	0.12
Lanolin	31.5	6	5.9	0.34
50	34.8	6.8	12.5	1.7
75	40.6	8	9.1	0.9
100	33.6	6.2	21.2	4.0
200	32	5.3	20.2	3.6

Figures

Fig. 1. Photographs of the root systems of compatible and incompatible rootstocks as viewed 20 days from grafting. A: non-grafted 'Arava' melon, B: 'Arava'/'Arava', C: 'Arava'/RS59 (compatible) and D: 'Arava'/RS62 (incompatible).

Fig. 2. Photographs of the grafting zone of the incompatible ('Arava'/RS62, A and C) and compatible ('Arava'/RS59, B and D) transplants after maintenance at high temperature conditions for 20 days. Note the degraded stem of the incompatible rootstock (white arrow).

Fig. 3. Auxin (IAA) concentration in the root and in the stem section (below the grafting zone) of the non-grafted melon ('Arava'), grafted compatible (Ar/Ar, Ar/RS59) and incompatible (Ar/RS62) transplants 20 days from grafting.

Fig. 4. The Effect of the auxin transport inhibitor, TIBA, pasted on the transplant's stem (above the grafting zone), on the development of root system in compatible ('Arava'/'Arava', Ar/RS59) and incompatible transplants (Ar/RS62).

Fig. 5. The effect of partial (half stem) and complete girdling, above the grafting zone of the incompatible Ar/RS62 transplants on the development of their root and shoot.

Fig. 6. Ethylene production by the roots of the different grafted transplants as affected by feeding with NAA at concentration of 10^{-6} M for 7 days in hydroponics.

Fig. 7. The effect of 10^{-6} M NAA, given to the root system, grown in hydroponics, on isozyme profile of soluble peroxidase in roots of the different grafted transplants.