

*COST Action FA1204*  
*1<sup>st</sup> Meeting*



*Program & Book of Abstracts*

Organized by  
*Agricultural University of Athens (AUA)*  
*Greece*



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## *Welcome note*

The Agricultural University of Athens (AUA) is delighted to host in its facilities the first meeting of the COST ACTION FA1204 on “Vegetable Grafting to Improve Yield and Fruit Quality under Biotic and Abiotic Stress Conditions”. As organizers of this meeting, we cordially welcome in AUA so many leading European scientists as well as younger scientists from several disciplines of Plant Sciences who address or are interested in including questions related to Grafting of Vegetables in their scientific research. The scope of this Action is to stimulate cutting-edge multidisciplinary collaborative research towards identifying and understanding how rootstock-mediated traits can improve vegetable crop yield and quality under biotic and abiotic adverse conditions. This first meeting is intended to provide an overview of current research activities on grafting of vegetables in Europe, as well as registering relevant research teams and their future research plans. A further goal of the first meeting of COST ACTION FA1204 is to set the bases of the work needed to implement the aims of the Action, and establish work plans within each working group. Last but not least, an objective of this first meeting is to provide the opportunity to European scientists from different disciplines who are working on grafting of vegetables, such as horticulturists, plant pathologists, plant breeders, plant physiologists and biotechnologists, to know each other, share knowledge and enhance scientific and technical collaboration. The collaboration between scientific groups from different European countries can be greatly facilitated within the COST ACTION FA1204 through mobility of scientists supported by the budget provided for Short Term Scientific Missions (STSM). The meeting will start with a general overview of the scope and the activities of the Action, given by the Chair. Subsequently, the Working Group (WG) leaders will shortly provide a state-of-the-art in the relevant scientific area and outline the aims and planned activities. Furthermore, the Editor and the STSM-coordinator will also shortly present the activities related to their functions. However, most of the meeting time is allocated into four sessions

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which include presentations of participants from the four Working Groups, as well as one session with presentations on general approaches relevant to vegetable grafting. The meeting will end up with a closing session devoted to general discussion and conclusions. We wish to all participants an excellent meeting and great days in Athens.

**The Chair of the Action & the Organizers**

**Giuseppe Colla, Dimitrios Savvas, Georgia Ntatsi & Andreas Ropokis**

## *Committees*

### *Organizing Committee*

*Dimitrios Savvas, Agricultural University of Athens, Greece*

*Georgia Ntatsi, Agricultural University of Athens, Greece*

*Andreas Ropokis, Agricultural University of Athens, Greece*

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### *Steering Group*

*Giuseppe Colla, University of Tuscia, Italy*

*Francisco Pérez Alfocea, CEBAS-CSIC, Spain*

*Andrew J. Thompson, Cranfield University, UK*

*Jan Henk Venema, University of Groningen, The Netherlands*

*Dietmar Schwarz, Institute of Vegetable and Ornamental Crops, Germany*

*Cherubino Leonardi, University of Catania, Italy*

*Dimitrios Savvas, Agricultural University of Athens, Greece*

*Georgia Ntatsi, Agricultural University of Athens, Greece*

*Working Group Leaders and co-Leaders*

**WG1**

*Andrew J. Thompson, Cranfield University, UK*  
*Halit Yetisir, University of Erciyes Melikgazi, Turkey*

**WG2**

*Jan Henk Venema, University of Groningen, The Netherlands*  
*Ian C. Dodd, Lancaster University, UK*

**WG3**

*Dietmar Schwarz, Institute of Vegetable and Ornamental Crops, Germany*  
*Roni Cohen, ARO, Neve Ya'ar Research Center, Israel*

**WG4**

*Cherubino Leonardi, University of Catania, Italy*  
*Carmina Gisbert (COMAV), Valencia, Spain*

***STSM coordinator***

*Dimitrios Savvas, Agricultural University of Athens, Greece*

***Editorial Board***

*Georgia Ntatsi, Agricultural University of Athens, Greece*  
*Mariateresa Cardarelli, University of Tuscia, Italy*

# Program

Monday 11 March, 2013		
<b>Symposium Opening</b>		
Chairpersons: <i>Giuseppe Colla &amp; Dimitrios Savvas</i>		
Time	Description	Speaker
8:00-9:00	Registration of participants	
9:00-9:30	Welcome and Introduction	<i>Giuseppe Colla</i>
9:30-9:45	Website/dissemination activities	<i>Georgia Ntatsi</i>
9:45-10:00	STSMs	<i>Dimitrios Savvas</i>
10:00-11:00	Presentation of the four Working Groups	<i>Anrew Thompson Jan Henk Venema Dietmar Schwarz Cherubino Leonardi</i>
<b>11:00-11:30 Coffee Break</b>		
<b>Section 1: Genetic resources and rootstock breeding</b>		
Chairpersons: <i>Andrew J. Thompson, &amp; Halit Yetisir</i>		
Time	Title	Speaker
11:30-11:40	Genetic resources and genomic tools for improving grafting efficiency in cucurbits and pepper	<b>Belen Picó (pp. 14)</b>
11:40-11:50	Melon and cardoon rootstock selection for the control of soilborne diseases	<b>Paola Crinò (pp. 15)</b>
11:50-12:00	Determination of rootstock potential of winter squash ( <i>C. maxima</i> duchesne) and pumpkin ( <i>C. moschata</i> duchesne) genotypes selected from black sea region for watermelon	<b>Ahmet Balkaya (pp. 16)</b>
12:00-12:10	Breeding of rootstock in cucumber	<b>Rana Kurum (pp. 18)</b>
12:10-12:20	Rootstock potential of turkish <i>Lagenaria siceraria</i> germplasm for watermelon: plant growth, yield and quality	<b>Halit Yetisir (pp. 19)</b>
12:20-12:30	Tomato genetic resources of Latvian origin	<b>Līga Lepse (pp. 20)</b>
12:30-12:40	The application of grafting technique in landraces of Solanaceae and Cucurbitaceae	<b>Penelope J. Bebeli (pp. 21)</b>
12:40-12:50	Activities carried out at the DISAFA-Plant Genetics and Breeding at the Univ. of Torino	<b>Lorenzo Barchi (pp. 22)</b>

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12:50-13:00	Root systems: the hidden half in grafted plants	Ana Maria Fita Fernández (pp. 23)
13:00-13:10	Recent situation in grafted vegetable seedling production of Turkey	Gölgen Bahar Öztekin (pp. 24)
13:10-13:20	A novel plant virus control method & grafting.	Andreas E. Voloudakis (pp. 25)
13:20-13:30	Metabolomics and genomics tools to assist vegetables grafting research and utilization	Yaakov Tadmor (pp. 26)
13:30-14:30	<b>Lunch Break</b>	
<b>Section 2: Rootstock-scion interactions and graft compatibility</b> <b>Chairpersons: Jan Henk Venema &amp; Ian C. Dodd</b>		
<b>Time</b>	<b>Title</b>	<b>Speaker</b>
14:30-14:40	Rootstock mediation of plant responses to drying soil by root-to-shoot ABA signalling?	Ian C. Dodd (pp. 28)
14:40-14:50	Preliminary studies of grafting cucumbers on Cucurbitaceae rootstocks and test of cross compatibility between <i>C. maxima</i> and <i>C. moschata</i>	Nikolay Velkov (pp. 29)
14:50-15:00	Grafting for the improvement of production and resource use efficiency on Solanaceous species in the Mediterranean basin	Francesco Giuffrida (pp. 30)
15:00-15:10	Systemic signalling in development and defence	Colin Turnbull (pp. 31)
15:10-15:20	Rootstock selection to improve nutrient-use efficiency and abiotic stress tolerance in tomato	Jan Henk Venema (pp. 32)
15:20-15:30	Molecular and physiological bases of the rootstock-scion interaction in fruit trees	Ana Pina (pp. 33)
15:30-15:40	Simultaneous analysis of major classes of plant hormones by HPLC-MS to explore root-to-shoot communication	Alfonso Albacete (pp. 34)
15:40-15:50	Improving establishment of grafted Cucurbitaceae transplants by directing root architecture and careful management in the field	Amnon Koren (pp. 35)
15:50-16:00	Splice grafting versus root pruning splice grafting - stand establishment and productivity issues in Cucurbitaceae vegetables	Astrit Balliu (pp. 36)
16:00-16:30	<b>Coffee Break</b>	

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<b>Section 3: Vegetable Grafting-General approaches</b> <b>Chairpersons: Giuseppe Colla &amp; Francisco Pérez-Alfocea</b>		
<b>Time</b>	<b>Title</b>	<b>Speaker</b>
16:30-16:40	Grafting as an environmentally and economically viable approach for sustainable vegetable production: ongoing and planned research studies	Sevilay Topcu (pp. 38)
16:40-16:50	Bulgarian research groups as partners in COST FA1204	Rossitza Rodeva (pp. 39)
16:50-17:00	Experiments with grafted vegetables in Slovenia - a review	Nina Kacjan Maršič (pp. 40)
17:00-17:10	Vegetable grafting as a research topic in Croatia	Katja Zanic (pp. 41)
17:10-17:20	Achievements and perspectives in research on vegetables grafting in Romania	Marian Bogoescu (pp. 42)
17:20-17:30	The vegetable grafting in Portugal and future prospects	Luis Miguel Brito (pp. 43)
17:30-17:40	Vegetable Grafting in Horizon 2020: new perspectives in EU research programme	Massimo Romanelli (ppt presentation)
17:40-19:00	<b>MC meeting</b>	

## Tuesday 12 March, 2013

### Section 4: *Rootstock-mediated resistance to biotic and abiotic stresses*

Chairpersons: *Dietmar Schwarz & Roni Cohen*

Time	Title	Speaker
8:00-8:10	Influence of grafting on physiological parameters under abiotic stress conditions	Angeles Calatayud (pp. 45)
8:10-8:20	Grafting vegetable crops to overcome abiotic stresses	Menahem Edelstein (pp. 46)
8:20-8:30	Grafting in melon to contrast fusarium wilt: a platform for host-pathogen interaction investigations	Alessandra Belisario (pp. 47)
8:30-8:40	Mechanism of abiotic and biotic stress tolerance in grafted vegetables	Hakan Aktas (pp. 48)
8:40-8:50	ROOTPOWER: Empowering root-targeted strategies to minimize abiotic stress impacts on horticultural crops	Francisco Pérez-Alfocea (pp. 49)
8:50-9:00	Grafting of vegetables improves tolerance against abiotic stresses	Dietmar Schwarz (pp. 51)
9:00-9:10	Effectiveness and durability of mi-resistant tomato cultivars and rootstocks to control root-knot nematodes	Francisco Javier Sorribas (pp. 54)
9:10-9:20	Grafting against abiotic stresses in vegetables crops	Yüksel Tüzel (pp. 55)
9:20-9:30	Grafted watermelon, melon and cucumber in isarel: agro-technology used for reducing soil-borne disease damage	Roni Cohen (pp. 56)
9:30-9:40	Response of grafted tomato to boron excess	Francesco Di Gioia (pp. 57)
9:40-9:50	Improvement of abiotic stress tolerance through the use of vegetable grafting	Giuseppe Colla (pp. 58)
9:50-10:00	Soil solarization in combination with grafting for the control of soil-borne plant pathogens	Polymnia Antoniou (pp. 59)
10:00-10:10	Effect of grafting on plant response to abiotic stress factors in cucurbits	Noémi Lukács (pp. 60)
10:10-10:20	Impact of grafting and rootstock genotype on mineral uptake by fruit vegetables	Dimitrios Savvas (pp. 61)
10:20-10:30	Emerging pest and diseases on grafted plants	Andrea Minuto (pp. 62)

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<b>10:30-11:00</b>			<b>Coffe Break</b>		
<b>Section 5: Rootstock-mediated improvement of fruit quality</b>					
<b>Chairpersons: Cherubino Leonardi &amp; Carmina Gisbert</b>					
<b>Time</b>	<b>Title</b>			<b>Speaker</b>	
<b>11:00-11:10</b>	Vegetable Grafting to Improve Yield and Fruit Quality under Biotic and Abiotic Stress Conditions			<b>Anja J. Dieleman (pp. 64)</b>	
<b>11:10-11:20</b>	Improving salt tolerance			<b>María José Asin (pp. 65)</b>	
<b>11:20-11:30</b>	Influence of grafting on fruit vegetables quality			<b>Alberto San Bautista (pp. 66)</b>	
<b>11:30-11:40</b>	Impact of grafting of tomato plants on fruit quality varies according to quality parameter			<b>Lieve Wittemans (pp. 67)</b>	
<b>11:40-11:50</b>	Quality of agricultural production as influenced by grafting			<b>Maria Dolores Raigón (pp. 68)</b>	
<b>11:50-12:00</b>	Improving grafting techniques and fruit quality assessment on eggplant and watermelon grafted plants			<b>Giovanni Iapichino (pp. 69)</b>	
<b>12:00-12:10</b>	Quality and postharvest performance of watermelon in response to grafting on cucurbit rootstocks			<b>Marios C. Kyriacou (pp. 70)</b>	
<b>12:10-12:20</b>	Optimizing Vitamin E and K production by grafting of tomato crop varieties			<b>Cédric Camps Felix Kessler (pp. 71)</b>	
<b>12:20-12:30</b>	Effects on yield and quality of grafted tomatoes with two pruning systems and organically amended with different composts			<b>Isabel Mourão (pp. 72)</b>	
<b>12:30-12:40</b>	Vegetable grafting to improve yield and fruit quality under biotic stress conditions at the COMAV institute			<b>Carmina Gisbert (pp. 73)</b>	
<b>12:40-12:50</b>	Vegetable grafting to improve yield and fruit quality under different Greek environmental conditions			<b>Ebrahim Avraam Khah (pp. 74)</b>	
<b>12:50-13:00</b>	Rootstock for organic fruity vegetables			<b>Justine Dewitte (pp. 76)</b>	
<b>Section 6: 1<sup>st</sup> Meeting FA1204: Closing</b>					
<b>Chairpersons: Giuseppe Colla &amp; Francisco Pérez-Alfocea</b>					
<b>13:00-14:00</b>			<b>Discussion and conclusions</b>		

# *Abstracts*

# *Section 1*

## *WG1*

### *Genetic resources and rootstock breeding*

## Genetic resources and genomic tools for improving grafting efficiency in cucurbits and pepper

Belen Picó\*, Cristina Esteras, Cristina Roig, Ana Fita, Alicia Sifres,  
Peio Ziarsolo, Jose Blanca, Joaquin Canizares, Carmina Gisbert

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The group of Cucurbits Breeding at the COMAV Institute (Universitat Politècnica de València) has constructed several core collections of cucurbits, including melons (*Cucumis melo* L.), other *Cucumis* spp and several *Cucurbita* species (*C. maxima*, *C. moschata*, *C. pepo* and *C. argyrosperma*), representing the genetic diversity of each species. In order to develop new and more efficient rootstocks, we are characterizing these collections for several traits of interest. We have selected some parentals according to their early and synchronized flowering and performed several interspecific crosses, selecting those combinations that give high fruit set and seed yields. Characterization of hypocotyls, root vigor and structure of the corresponding hybrids and also resistance to different soil pathogens is also being performed. The interaction of the Cucurbits Breeding Group with the Bioinformatic and Genomics Group has also provided a high number of genomic tools in *Cucumis* and *Cucurbita*. We have produced a complete transcriptome of the species, a SNP high throughput genotyping platform, a SNP-based genetic map, and several interesting mapping populations (RILs and ILs). All these tools (most available at <http://www.cucurbigene.net/> and <http://www.melogene.net/>) will be useful to better study genetic factors involved in grafting compatibility and efficiency. In pepper, the group 'Development of Rootstocks for Vegetable Grafting' has evaluated the host suitability of pepper genotypes of *C. annuum* and the related species *C. chinense*, *C. frutescens* and *C. pubescens* to *Meloidogyne incognita* and assessed the presence/absence of resistant genes using PCR-specific markers.

## Melon and cardoon rootstock selection for the control of soilborne diseases

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ENEA activities are focused on the development and selection of soilborne disease germplasm to be used as rootstocks in grafting combinations. The species of interest are melon and globe artichoke, both lacking of commercial materials effectively resistant to *Fusarium oxysporum* f. sp. *melonis* race 1,2 and *Didymella bryoniae* (melon) as well as to *Verticillium dahliae* (globe artichoke).

Breeding and selection for disease resistance is the expertise of ENEA in grafting technology; in addition, it interacts in a complementary way with other institutions (Universities of Viterbo, Catania and Messina and CRA-RSP) to achieve the final goal of having grafted plants capable of controlling diseases, also being productive and qualitatively appreciable. Melon plants grafted on the *Cucurbita* rootstock RS 841, resistant both to fusarium wilt and gummy stem blight, produce high yields and fruit quality also under stress conditions. Only very few accessions of wild cardoon were found resistant to *V. dahliae* and therefore usable in grafting programs. In this case, different grafting techniques have been compared and the grafted plants have been analyzed either in the field, for productivity and quality, or in the lab, for compatibility and physiological studies on rootstock/scion union formation. Inbred lines of cardoon are being developed.

## Determination of rootstock potential of winter squash (*C. maxima* duchesne) and pumpkin (*C. moschata* duchesne) genotypes selected from Black Sea region for watermelon

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The most currently used rootstocks for grafted watermelon seedling production are *C. maxima* × *C. moschata* interspecific hybrid, *Lagenaria siceraria*, *Cucurbita* spp. (winter squash and pumpkin) with *Benincasa* sp. and *Citrullus* sp. species. All rootstocks used in grafted seedlings production in Turkey are imported from foreign countries. Pumpkin and winter squash are two important cucurbit species in Turkey. This breeding study was carried out to develop a domestic rootstock for watermelon using Turkish winter squash and pumpkin genetic resources. The present study was conducted between 2008-2011 in Samsun Province. Primarily, the parents (K11, K12, K14 and B8) were selected for interspecific crossing by selection method. The promising hybrids for seed yields were determined after interspecific breeding. After the selection studies, 14 winter squash, 9 pumpkin and 7 interspecific hybrid genotypes were determined as resistant to *Fusarium oxysporum* f. sp. *niveum* (0, 1 and 2 races) and found to be sensitive to root knot nematode (*Meloidogyne incognita* 2 races). Grafting success of rootstocks differed between 35.33-96.63%. It was determined that the grafting success of K10 winter squash genotype, M2 and M3 interspecific genotypes with Crisby F<sub>1</sub> watermelon cultivar were the highest. The grafted watermelon genotypes showed higher vegetative growth than ungrafted control plants seedlings. For the last year of the research; total yield, fruit weight, fruit length and diameter, total soluble solids (TSS), fruit flesh color, fruit firmness, lycopene and vitamin C content and some aromatic substances were also determined to search out the effect of rootstocks on the quality and yield components of watermelon. Grafting had a positive effect on TSS and fruit firmness. Fruit flesh colour, lycopene, vitamin C and aromatic substances differed with the rootstocks. The stock/scion combinations of M2/Crisby, M5/Crisby and M3/Crisby were found to be superior for yield, quality and some other characteristics than ungrafted and other rootstock/scion combinations. They also gave the

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equal and good results as commercial watermelon rootstocks used currently. The M2 and M5 hybrid cucurbit rootstocks were found to be candidate for watermelon rootstock.

## Breeding of rootstock in cucumber

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Vegetable grafting was first started in 1998 with 70.000 seedlings in Turkey. The number of nursery in Turkey was 12 in 1998. Two of them produced grafted seedlings. Nowadays, the number of nursery is 85. 19 of them produce grafted seedlings and 109 million seedlings were grafted. Grafted watermelon seedlings are used the most in our country because of yield increase . Addition to this, grafting is to control soil-borne diseases such as fusarium and verticillium. Especially, soil-borne diseases are common in The Black Sea region of our country and rootstocks used by growers are foreign hybrid cultivars. There are no rootstocks bred in Turkey for cucumber. From this point the aim of the project is to develop Fusarium resistant hybrid rootstock for cucumber. With this project, 74 materials were collected from different regions of Turkey. Used species are *C.maxima*, *C.moschata*, *C.pepo* and *Lagenaria* spp. These materials were selfed two generations. In later stages of the study, pure lines to be obtained will be tested against Fusarium . Commercial varieties will be grafted on resistant rootstock hybrids.

## Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: plant growth, yield and quality

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In this study, rootstock potential of bottle gourds (*Lagenaria siceraria*) collected from Mediterranean region was investigated. Crimson Tide watermelon cultivar was used as scion and 2 commercial rootstocks were also used for comparison. Survival rate of grafted plant and effect of rootstocks on plant development were determined. In addition, emergence performance of rootstocks was studied at 3 different temperatures ( $14\pm 1$ ,  $18\pm 1$ ,  $24\pm 1$  °C). All grafted plants (control, grafted) were tested against 3 known race of *Fon*. In open field experiment, grafted plants were planted at early spring and effects of rootstocks on yield and fruit quality were determined.

Survival rate of grafted plants varied from %78 to %100. Grafted plant on both commercial rootstocks and local rootstocks showed higher growth performance than control plants. Increase in plant fresh weight ranged from 47% to 253%. While all genotypes showed well emergence at  $24\pm 1$  °C, emergence time and rate were negatively affected by decreasing temperature. At  $14\pm 1$  °C, emergence was not observed in most of the rootstocks while limited emergence was observed in eight rootstocks.

All grafted plant produced higher yield than control plants. Local rootstocks had higher yield than commercial rootstocks. Among local bottle gourds genotypes, 01-16, 07-42, 07-45, 31-09, 31-15 and 46-03 were found promising as regarded to yield.

Quality parameters of fruits harvested from grafted plant and control plants were found similar. it was concluded that bottle gourds genotypes collected from Mediterranean region had high rootstocks potential for watermelon.

## Tomato genetic resources of Latvian origin

Līga Lepse

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Pūre Horticultural Research Centre (Pūre HRC) is the institution in Latvia, responsible for maintenance of vegetable genetic resources. The tomato seed collection is preserved in Gene Bank of Cultivated Plants. Active collections are maintained in Pūre HRC.

Tomato in Latvia was introduced at the end of 19 century and now it is among the most popular vegetables grown in Latvia, although the climatic conditions are poorly favourable for tomato growing in open field conditions. This is the reason why mostly local cultivars are developed for indoor growing (most often in plastic tunnels). Three indeterminant cultivars are registered in Latvia: `Jūrmala`, `Tīraines` and `Kondīne uzlabotā`. Also one outdoor variety is originated in Latvia - `Pūres konservu`. Some cultivars are developed/grown by hobby gardeners. The most popular are `Cēsu agrais`, `Vidzemes karalis` and `Gaujmalas`. These cultivars are not used or investigated with purpose for using in grafting till now.

In commercial tomato production in glasshouses commercial grafted tomatoes are imported, mostly grafted on rootstock `Maxifort`.

## The application of grafting technique in landraces of Solanaceae and Cucurbitaceae

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Landraces are heterogeneous, genetically rich dynamic populations. Even though have been considered as an interesting genepool for breeding many useful traits they have not yet been fully exploited. Landraces frequently have distinctive organoleptic values and can produce value-added products for farmers due to their increased popularity in certain markets.

Landraces could be used either as rootstocks or as scions. They have been tested as rootstocks with various results depending on the genetic material. For example *L. siceraria* landraces have showed a high rootstock potential for watermelon while landrace of *C. moschata* has been a promising source of breeding material for rootstocks of honeydew melons. On the other hand tomato landraces showed susceptibility to *Meloidogyne javanica* when used as rootstocks. Landraces can also be used as scions when their susceptibility to soil borne diseases is a problem. In this case grafting can be applied to unite the disease resistance of a hybrid cultivar and the high fruit quality of a landrace.

A short review on the use of landraces as rootstocks that has shown various results will be presented. Greece has a wealth of landraces both in Solanaceae and Cucurbitaceae family that have been barely screened as rootstocks or scions. Possibilities of using this diverse germplasm by exploiting the advantages of grafting technique will be discussed.

## Activities carried out at the DISAFA-Plant Genetics and Breeding at the University of Torino

Lorenzo Barchi\*, Ezio Portis, Alberto Acquadro and Sergio Lanteri

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The Plant Genetics and Breeding group at the University of Torino has acquired a sound experience in the development and application of molecular markers for genomic analyses of horticultural species.

In pepper (*Capsicum annuum* L.), a high resolution intraspecific linkage map was developed for the identification of QTLs related to key agronomic traits and resistance to *Phytophthora capsici* and *P. parasitica*.

In eggplant (*Solanum melongena* L.), EST-based microsatellite markers were developed for the construction of an intraspecific linkage map, hereafter improved by applying SNP markers identified by combining RAD TAG technique with NGS technologies. The high saturated map made it possible to detect QTLs related to anthocyanin pigmentation.

Recently the group has focused its efforts on eggplant genome wide transcriptional profiling (RNA-seq) in response to biotic (*Verticillium* spp. and thrips) and abiotic (drought) stresses for identifying key genes involved.

Within the COST action, the Plant Genetics and Breeding group can provide expertise for molecular assessment of genetic compatibility between graft and rootstock, as well as for unrevealing the genetic mechanism involved in the grafting aimed at improving yield and fruit quality under biotic and abiotic stress conditions.

## Root systems: the hidden half in grafted plants

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Grafting joins together in one plant: i) traits of a high value variety(graft) and ii) tolerance to soil biotic and abiotic stresses (rootstock).Undoubtedly, root systems play a central role in the rootstock performance. However, the trend when doing vegetable grafting is to study the final result of a rootstock-graft combination instead of studying which genotypes could behave better rootstocks because of their roots (except for biotic resistances). This is mainly due to the difficulty in phenotyping the root systems. There are two main ways to evaluate root systems: i) studying roots *in situ* (e.g. rizhotrons, minirizhotrons, magnetic resonance) or ii) studying roots by extracting them from the media (usually soil). Both ways include many different methods and present pros and cons. In our lab we have a large experience in phenotyping vegetable root systems. We have used, among others, special agar plates designed by us to identify melon root architecture QTLs, pot root extraction to evaluate the rootstocks development, paper supported hydroponics to study the diversity in root systems within melon and pepper varieties, hydroponics to relate root development and P-starving responding genes expression, and lately we are successfully using field extraction to phenotype root systems of diverse pepper accessions. This experience can be used for better screen rootstocks.

## Recent Situation in Grafted Vegetable Seedling Production of Turkey

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Grafting of vegetable seedlings is a technique which has been started to be used especially to provide resistance to soil borne pathogens; to increase water and nutrient uptake due to the vigorous root system of rootstocks resulting an increase in plant growth, yield and fruit quality and improving the tolerance against abiotic stresses.

In Turkey grafted vegetable seedling started to be used commercially in 1998 in Antalya. At the end 1990s there was only two nurseries producing grafted tomato seedling and the amount was around 500 thousand seedling. From 1998 until now, Turkey has showed a great development in this regard and nowadays 17 big nurseries are produced grafted seedling namely tomato, watermelon, eggplant, pepper and cucumber. It is estimated that approximately 110 million grafted seedling (47 million watermelon, 42 million tomato, 7 million eggplant, 5 million pepper, 5 million cucumber and 0.5 million melon) was produced in the spring season of 2012. Turkey also exports watermelon and cucumber seedlings to Romania, Hungary and Georgia in small amounts. Grafted watermelon seedlings are mainly used in south region of Turkey, especially in Adana for the production under low plastic tunnels however it is expected a 25% decrease due to the financial problems.

In this presentation a brief information will be given related to current grafted seedling production and present problems of nurseries.

## A novel plant virus control method & grafting

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A novel method to induce resistance against *Cucumber mosaic virus* (CMV) by applying exogenously *in vitro* or *in vivo* produced double-stranded RNA (dsRNA) molecules has been developed and employed successfully against CMV in tobacco<sup>3</sup> and tomato. Such dsRNA molecules, the short interfering RNAs (siRNAs) and the artificial micro RNAs (amiRNAs) could induce the RNA interference (RNAi) machinery of the plant against viral infections via a post-transcriptional degradation silencing mechanism. Upon deep sequencing of small RNAs from tissue infected with CMV it was determined that high siRNA-producing regions (hot spots) and low siRNA-producing regions (cold spots) exist in the CMV genome, information useful for the selection of the most appropriate siRNA to be used as an RNAi inducer molecule.

Small RNAs (especially siRNAs) act non-cell autonomously with supporting evidence coming from micro-grafting experiments that indicated a sequence-specific silencing signal moving from transgene-silenced rootstock towards a non-silenced scion<sup>5</sup>. More recently, grafting experiments in *Arabidopsis thaliana* followed by deep sequencing analysis indicated that small RNAs were shown to be transported from rootstock to scion<sup>1,4</sup>, directing epigenetic modification in the recipient cells<sup>4</sup>. Such an epigenetic control of gene expression could be of significant importance for scion development as well as tolerance to biotic and abiotic stresses, if the responsible gene is targeted. In a recent study in apple trees, it was shown that environment could influence the transport of mobile signals<sup>2</sup>, suggesting that environmental effects need to be studied before such a method of gene expression control is considered for agricultural application.

Lastly, it should be noted that EU considers that the product (i.e fruit) deriving from a nontransgenic scion grafted on a transgenic rootstock is transgenic. Therefore, nontransgenic means, such as the above-mentioned RNA-based vaccination method, able to induce desirable epigenetic modification(s) need to be developed.

## **Metabolomics and genomics tools to assist vegetables grafting research and utilization**

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Grafting involves the physical contact and interaction of heterologous root and shoot systems. A major concern to vegetable grafting is the effect of rootstock, which in certain cases negatively affect fruit quality. Rootstock can affect several quality traits including fruit size, shape, texture, taste, color and nutritional value. Thus, metabolites and signals (hormones and RNA) from each interacting systems that affect the performance of other system are of high importance. My laboratory is equipped with HPLC, LCMS and all necessary equipment for molecular biology analyses and specializes in the utilization of metabolomics and genomic tools to identify genes and compounds associated with fruit quality, especially in melons and watermelons. My activities could be combined with grafting technologies to establish a system approach to study the effect of grafting on fruit quality and plant performance

## *Section 2*

### **WG2**

## *Rootstock-scion interactions and graft compatibility*

## Rootstock mediation of plant responses to drying soil by root-to-shoot ABA signalling?

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The use of wild tomato relatives (*Solanum spp.*) as rootstocks provides an opportunity to introduce stress tolerance into the domestic tomato (*Solanum lycopersicum*), and to evaluate the role of root-supplied phytohormones (such as abscisic acid, ABA) in regulating growth and water use of an economically important crop. Several experiments investigated rootstock effects on leaf water potential and xylem ABA concentration of plants exposed to mild soil drying (soil water potential between -10 and -100 kPa). *S. lycopersicum* (cv. Ailsa Craig) grafted on rootstocks of the drought-tolerant wild species *Solanum pennelli* had a lower leaf area and leaf water potential, and higher xylem ABA concentration than those plants grafted on a *S. lycopersicum* rootstock. A commercial tomato cultivar (Boludo F1, Seminis Vegetable Seeds) was grafted onto rootstocks from a population of 110 recombinant inbred lines (RILs) derived from a *S. lycopersicum* var. *cerasiforme* x *S. pimpinellifolium* cross, and grown under well watered conditions or mild soil drying. There was a 2-fold rootstock-mediated variation in shoot fresh weight, with some rootstocks being relatively more vigorous under well watered conditions. Further analysis of leaf water potential and xylem ABA concentration within this population is ongoing.

## **Preliminary studies of grafting cucumbers on *Cucurbitaceae* rootstocks and test of cross compatibility between *C. maxima* and *C. moschata***

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Preliminary studies were performed concerning grafting cucumbers on different cucurbit rootstocks in Bulgaria. The trials were carried out in greenhouse conditions at “Maritsa” Vegetable Crops Research Institute – Plovdiv during 2011-2012. It was established that cucumber cultivars successfully can be grafted on *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita maxima* x *Cucurbita moschata* F1, *Lagenaria siceraria* and *Luffa cylindrica*. The percentage of success grafted plants varied depending on used rootstock.

It was investigated the effect of grafting on elements of productivity and the yields of three long type cucumber cultivars. The data revealed specific interactions between cucumber cultivars and used rootstocks. The highest yield was recorded in cv. Kiara F1 grafted on *Cucurbita maxima* x *Cucurbita moschata* F1 rootstock – 122% compared to non grafted one.

It was performed organoleptic evaluation on fruits between grafted and non grafted plants. Cluster analysis shows close similarities of fruit sensory traits between non grafted plants and these grafted on *Cucurbita maxima* x *Cucurbita moschata* F1 rootstock.

In order to obtain new interspecies cucurbit hybrids it was tested cross compatibility between *C. maxima* and *C. moschata* cultigens. Most of performed crosses failed but five interspecies hybrids were only obtained. Studies should be continued with cultigens that possess female type of flowering or male sterility.

## **Grafting for the improvement of production and resource use efficiency on Solanaceous species in the Mediterranean basin**

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Sicily is one of the most important regions for greenhouse cultivation in the Mediterranean basin. Among the alternatives to methyl bromide once used in the protected cultivation, grafting is increasing its importance for Solanaceous and Cucurbitaceous crops. Almost the 80-90% of eggplant, 40-50% of melon and watermelon, 30-40% of tomato plants for protected cultivation are grafted in Sicily; negligible is the use of grafted pepper. The genotypes proposed as rootstock by the seed companies are mainly offered being resistant to specific diseases. Limited attention is given to abiotic stresses response and to nutrient uptake, although protected cultivation in the basin of Mediterranean is often characterized by the use of high salinity water and/or not optimal fertilization strategies.

The research activity carried out at DISPA in the last decade was mainly focused on the effects of grafting on yield, quality and nutrient use efficiency of Solanaceous species. The effect of salinity and nutrient solution composition in relation to grafting combinations was also investigated. The study of the mechanisms explaining the observed interactions between scion and rootstock could allow the improvement of nutrient use efficiency and the reduction of the negative effects of salinity on Solanaceous crops by using grafted plants. For this purpose, the applied research of DISPA should be integrated with fundamental studies.

## Systemic signalling in development and defence

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We work on fundamental mechanisms of long-distance signalling in a range of model and crop species, and especially on mobile molecules that regulate development or alter plant immunity. We developed the micro-grafting platform for Arabidopsis seedlings that has been widely adopted by other laboratories. In our hands, Arabidopsis grafting has revealed (a) the conservation of strigolactone functions in rootstocks that regulate shoot branching architecture, (b) that shoots send signals to roots to regulate xylem cytokinin flux and (c) that the FT protein is a mobile hormone that transmits from source leaves to sink meristems to govern flowering time. In the pea aphid – *Medicago truncatula* model, we have used grafting to show that inducible and systemic immunity are dependent on both the aphid biotype and the host resistance genotype, with enhanced immunity only seen in combinations of avirulent aphids on hosts carrying a cognate R gene. We aim to resolve the difference between gene-for-gene dependent specific resistance and broad spectrum stress and defence signalling, including cross-protection across multiple stresses. Current work is comparing the proteomes of the dual phloem systems of cucurbits, and testing for further systemic signal components in Arabidopsis.

## Rootstock selection to improve nutrient-use efficiency and abiotic stress tolerance in tomato

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This project explores the possibilities to apply rootstocks as a tool to improve the nutrient-use efficiency and abiotic stress tolerance of tomato. The selection and breeding of such robust rootstocks is difficult since the identified Quantitative Trait Loci (QTLs) of these complex traits have hardly any value for practical breeding purposes. In addition, the knowledge about the physiology behind a successful rootstock (root-shoot interaction) is very limited. This project, therefore, aims to identify the key-physiological characteristics that reflect the complex underlying genetic make-up of the desired root-derived traits. Within this project a set of inter-specific hybrids was generated which contrast in low-temperature tolerance and vigour. These hybrids are currently tested as rootstock in grafting studies under different growth conditions. The interactions between rootstock and scion under these conditions are studied on the level of the root (morphology and architecture, hydraulic conductance, water and nutrient uptake), root-shoot signalling (xylem sap composition and pH) and scion (leaf morphology, development, growth, photosynthesis, stomatal conductance and fruit production). One of the main targets is to unravel the underlying hormonal regulations. The knowledge generated will finally be used to develop a reliable high-throughput screening method which supports the selection of vigorous robust rootstocks for tomato.

## **Molecular and physiological bases of the rootstock-scion interaction in fruit trees**

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Plant grafting is a widely used means of plant propagation. It is of considerable importance in the deployment and regional adaptation of elite cultivars, but its range of application is restricted by anatomical, physiological and biochemical aspects that produce incompatible grafts. The localized type of graft incompatibility is manifested by physical breaking of the trees at the point of union several years after grafting. It is a general phenomenon in many cultivated fruit species and is a factor that makes rootstock and cultivar selection difficult because the introduction of some new varieties requires knowledge of the extent and nature of incompatibility reactions. For a deeper knowledge of this topic, the main goal of our group is to identify genes differentially expressed and anatomical markers involved in the early stages of the incompatibility reaction between graft partners, potentially affecting the fate of the graft. These studies will contribute to a genomic and cellular-level understanding of the complex process of graft incompatibility and thereby ameliorate incompatibility and to extend the range of useful and compatible fruit tree grafting.

## **Simultaneous analysis of major classes of plant hormones by HPLC-MS to explore root-to-shoot communication**

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During the last 20 years, it has been demonstrated the importance of the root-to-shoot communication in the development of chemical and hydraulic signals as an adaptive response of the plant to environmental stresses, highlighting the primary role of phytohormones. Additionally, the interaction with soil biota could also affect such communication. Grafting is an excellent tool to explore and to exploit root-to-shoot communication, both in terms of shoot response capacity and root hormone biosynthesis, in order to improve abiotic stress tolerance. Analysis of phytohormones is therefore essential, and as these compounds are present in the plant at very low concentrations, an accurate and efficient analytical method with simultaneous quantitative profiling of multiple hormone classes is required. Conventional techniques (immunoassays, flame ionization, UV, fluorescence or electrochemical detection) usually require significant amounts of solvent, time and labor and are limited by sensitivity and specificity. We have developed a simple, high-throughput, sensitive analytical HPLC-MS method that requires limited sample material (50-100 mg of plant tissue or 50-100 µl of xylem sap) to quantify all major hormone classes and precursors in a single run: cytokinins (zetatin, zeatin riboside, isopentenyl adenine), gibberellins (GA1, GA3, GA4), indolacetic acid, abscisic acid, jasmonic acid, salicylic acid, and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid.

## Improving establishment of grafted Cucurbitaceae transplants by directing root architecture and careful management in the field

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Grafted cucurbits have been used in Israel for 15 years. Grafting expanded following the ban on methyl bromide for soil disinfestation. In general, the performance of grafted transplants meets growers' needs, however, the summer and fall seasons still pose some difficulties, such as sensitivity of the *Cucurbita* rootstock roots to high soil temperature. Root-temperature susceptibility can cause increased sensitivity to soil-borne pathogens such as *Macrophomina phaseolina*.

Good performance of grafted plants starts in the nursery and continues later on in the field. Directing root architecture in the nursery and building strong root systems are key factors in the production of healthy plants. These goals can be achieved by using the correct shape and size in the planting tray. In the field there is a need to reduce soil temperature by using yellow plastic for bed mulching rather than transparent plastic. In addition, there is a need to design the correct irrigation program,. . Vigorous transplants with robust root systems can better cope with soil-borne pathogens. One of the most dangerous is *Cucumber green mottle mosaic virus* (CGMMV), which attacks cucumber, melon and watermelon. Results have shown that healthy root systems can indeed significantly contribute to better performance of the plant in disease-infested soil.

## **Splice grafting versus root pruning splice grafting - stand establishment and productivity issues in Cucurbitaceae vegetables**

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Two different grafting methods; splice grafting (SG) and root pruned splice grafting (RPSG) were applied in watermelon and cucumber grafted seedlings. Compared to RPSG seedlings, a higher plant dry matter and larger leaf area per plant were recorded for SG seedlings at the end of nursery stage. Despite that, a significantly higher relative growth rate was found for RPSG seedlings, mostly due to a higher net assimilation rate. Most of plant assimilates were allocated to the growth of new root system. The same advantage was maintained by RPSG seedlings after transplanting. As a result, a faster establishment rate was recorded. Generally speaking, the stand establishment rate of transplanted seedlings was drastically reduced due to the increase of nutrient solution salinity, but still, significantly higher values were recorded in case of root pruned splice grafted seedlings. A higher concentration of plant growth hormones was indicated by a higher rooting index of *Phaseolus acutifolius*, in case of RPSG seedlings, and to that was attributed the significantly higher stand establishment rate of them versus SG seedlings. Thanks to the higher stand establishment rate, a significantly higher harvesting rate and a significantly higher fruit production was recorded for RPSG seedlings.

## *Section 3*

### *Vegetable Grafting*

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### *General approaches*

## **Grafting as an environmentally and economically viable approach for sustainable vegetable production: *ongoing and planned research studies***

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Cukurova Region supplies about 85% of the country's vegetable production, particularly watermelon and tomato grown under low and high plastic tunnels. Due to rise in temperature and decrease in precipitation during the last few decades, drought has become a recurring phenomenon which has also led to crop failure and economic losses. Vegetable production in Cukurova is intensively managed (high inputs of fertilizers, water and pesticides) and results in pollution of resources. Enhancing yield and improving crop tolerance to abiotic stresses, vegetable grafting may also provide an environmentally-friendly approach to optimizing resource use. Owing to its multifaceted benefits, grafting has been increasingly used in the production of solanaceous and cucurbit vegetables in Turkey with a view to disease control, plant vigour, yield increase and improved fruit quality during the last two decades.

Considering the above mentioned constraints and the opportunities for the vegetable production in Turkey, a brief summary of our on-going and planned research projects on assessment of effects of grafting with vigorous rootstocks on plant growth, fruit yield and quality as well as water and fertiliser use efficiency will be presented in this meeting.

## Bulgarian research groups as partners in COST FA1204

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Bulgaria has very favorable climatic conditions and long year traditions in breeding and production of vegetable crops. Some biotic and abiotic stress factors are important constraints in production resulting in reduced and compromised yield. Fungal diseases affecting root/stem base are the most frequently encountered problem for vegetables damaging the plants at any stage. Some of achievements in studying different aspects of vegetable crops in Bulgaria are presented. Institute of Plant Physiology and Genetics (IPPG) - Sofia and Maritsa Vegetable Crops Research Institute (VCRI) - Plovdiv are mainly envisaged. Activities related to WG3 that could be performed by different research groups of IPPG and VCRI are outlined. As main biotic stress factors some soil fungal pathogens and nematodes will be involved. Analysis of changes in photosynthetic activity of the grafted and non-grafted vegetable plants in norm and after different stress treatments will be performed. The content of photosynthetic pigments will be determined. Photochemical efficiency of photosystem I and photosystem II will be defined and the integrity of membranes will be estimated. The effect of grafting on nitrogen and water use efficiency under normal and stress conditions could be evaluated by different analyses.

## Experiments with grafted vegetables in Slovenia – a review

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The experiments of vegetable grafting were started in Slovenia in 2005 and were carried out at the Biotechnical Faculty, University of Ljubljana. The aims of the experiments were: (1) to evaluate the yield quality and physiological response of grafted plants on different abiotic stresses; (2) to evaluate the influence of different rootstocks on the quantity and quality of the yield. In grafted tomato experiments, (lasted from 2006 till 2010) tomato varieties, important for local producers were grafted onto the rootstocks, which were received from different seed companies. In 2011, fruit quality parameters of grafted tomato, in response to salt stress were evaluated. Experiments with grafted eggplants, (2007 till 2009), were conducted in the open field and in the greenhouse, where hybrid and local varieties were included and grafted onto the tomato rootstocks. The eggplant yield and fruit quality compounds were evaluated. Grafted cucurbits experiments were conducted in 2005, 2008, 2010, and 2012 with watermelon and mini watermelon, grafted onto different rootstocks. Fruit yield and quality parameters were evaluated. In 2006, 2009 and 2011, the experiments with grafted cucumbers, grown in conventional and hydroponic systems were carried out, to evaluate the yield and fruits quality.

## Vegetable grafting as a research topic in Croatia

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Grafting is a common practice in watermelon field cropping with the purpose of environmental stressors mitigation. This technique is not widespread in other vegetable species, although there are some occasional good examples in tomato or cucumber greenhouse cropping, but based on seedlings import.

In our previously researches, the role of rootstocks on mitigation of biotic and abiotic stressors in cucurbits were studied. Thus in the trials on grafted cucumber in soil infested with root-knot nematodes, vegetative growth and yield of the plants grafted on the interspecific hybrid rootstocks, Strong Tosa and RS 841 Improved, were the highest. Based on the study in watermelon, rootstock Strong Tosa was selected in withstand of salt stress probably due to the ability to induce anatomical adaptation and superoxide dismutase activity in response to salt stress.

During the current study, the effect of grafting and nitrogen rate on yield and aerial pests populations has been studied in hydroponics tomato. Tomato yield was not affected by rootstock Arnold, while population density of *Bemisia tabaci* and *Tuta absoluta* was lower on grafted plants. The study has been continued, composition of amino acids in phloem sap, and fruit quality of grafted and ungrafted plants will be analysed.

## **Achievements and perspectives in research on vegetables grafting in Romania**

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Since crop rotation is rarely adopted in greenhouses or solar, the reduction of yield progressively affects the crops; soil-borne pathogens and nematodes are very destructive in vegetable crops and one of the most limiting factors to farmer's income, thus making necessary the adoption of soil disinfection practices. Methyl bromide was and until now remained probably the only fumigant that could effectively be used against nematodes, weeds, pathogens, insects and rodents. About 107.71 tons of methyl bromide has been used in 2003 in the agricultural sector in Romania. But, according to the Montreal Protocol, the use of methyl bromide for soil treatment aimed at plant protection (fumigation) in Romania, is banned since 2005 January 1st. In this context, beginning in year 2003, research was performed which was aimed at establishing some alternatives to the use of methyl bromide in Romanian horticulture. Between the non-chemical alternatives that have been evaluated and can be used in specific conditions of Romania, the benefits of using grafted plants in growing vegetables outweigh the possible risks. The aim of this paper is to describe the achievements and perspectives in research on vegetables grafting in Romania.

## The vegetable grafting in Portugal and future prospects

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The first trials of grafting on tomato plants in Portugal took place in 1999, followed by watermelon and cucumber in 2002. However, the commercial tomato production based on grafted plants started only in 2007. Since then, the demand for grafted plants for protected crops highly increased. In Portugal, in 2008, the total area of tomato crop either for fresh consumption or for processing was about 13,000 ha, representing 1,100,000 t, and today, more than half of the area for fresh tomato is based on grafted plants. Most of these plants arrive from Spain but the production of grafted tomato plants increased fast in Portuguese nurseries in the last three years. Apart from tomato, the production of grafted nursery plants as well as the production of vegetable crops, either inside greenhouses or outdoor, based on grafted plants, is rapidly increasing for cucumber, pepper, melon, water melon and green beans. Research on vegetable grafting is mainly related to rootstock resistance concerning *Pyrenochaeta lycopersici*, *Fusarium oxysporum* and *Meloidogyne* spp. as well as to pruning systems, including its effects on tomato yield and quality.

## *Section 4*

### **WG3**

## *Rootstock-mediated resistance to biotic and abiotic stresses*

## **Influence of grafting on physiological parameters under abiotic stress conditions**

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Our work is focused in understanding the physiological mechanisms that confer pepper grafted plants resistance to salinity and water stresses that can result in alteration of plant performance and at the end decrease both yield and product quality. To reach this goal, different research lines are tackled.

One is to identify rootstocks from germplasm accessions and commercial cultivars which have traits that resist salinity and water stresses. For this evaluation, we search for physiological parameters that permit distinguish between tolerant and sensitive rootstocks. Photosynthesis performance has shown to be one of the most robust parameter to select resistant rootstocks.

A good compatibility between rootstock and scion is required to achieve abiotic stress resistance. Previous studies in melon grafted plants have demonstrated that chlorophyll fluorescence imaging is a sensitive, quickly and non-invasive technology for monitoring compatibility status. Now, we are evaluating the applicability of this technique for scion-rootstock connection diagnostic in pepper grafted plants.

We are also evaluating the influence of rootstocks and scions on physiological disturbances that are induced by salinity and water stresses, considering the mechanisms to strengthen the resistance to stress such as photosynthesis behaviour, antioxidant systems, nutrient and water uptake, osmotic adjustment or enzymes activities.

## Grafting vegetable crops to overcome abiotic stresses

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We started grafting activity of cucurbit crops fifteen years ago. Our group includes a phytopathologist, a breeder, a physiologist, and technical staff. Our work is devoted to investigating horticultural and physiological aspects related to rootstock-scion compatibility, and abiotic stresses. This research, accompanied by the availability of a large pool of cucurbit germplasm, led us to conduct a breeding program aimed at developing rootstocks suitable for abiotic stresses. We have excellent facilities at the Neve Ya'ar Research Center and Volcani Center. These include research plots, climate-controlled greenhouses, growth chambers and mist beds, and equipment and expertise for grafting and follow-up physiological and horticultural investigations.

Projects I am involved with include:

- Use of grafting to mitigate chemical stresses in vegetables under arid and semi-arid conditions.
- Rootstock delivery of PGIPs (polygalacturonase inhibiting proteins) for pathogen protection in grafted tomatoes.
- Identification of anatomical, physiological and biochemical factors involved in compatibility of rootstock-scion in vegetable crops.
- Wild watermelons as potential rootstocks for intra-specific grafting of watermelon.
- Effect of grafting on fruit quality.

## Grafting in melon to contrast fusarium wilt: a platform for host-pathogen interaction investigations

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Melons are grafted mainly to control *Fusarium* wilt, a devastating disease caused by *Fusarium oxysporum* f. sp. *melonis* (FOM). Four races of the pathogen (0, 1, 2 and 1,2) have been defined according to host resistance genes overcome by variants of the pathogen. FOM race 1,2 is the most virulent and widely present in Europe. Though grafting is a widely applied system in commercial melon cultivation, knowledge is lacking on its effects on the pathogen vascular colonization. On this account, we investigated both grafted and ungrafted plants considering the compatible and incompatible host pathogen interaction using FOM race 1 or FOM race 1,2 as inoculum. Pathogen development inside grafted and ungrafted melon genotypes was compared using qPCR and isolations from stem tissues. Results showed that fungal development was highly affected by host-pathogen interaction (compatible/incompatible) and time (days post inoculation). The principal effect on fungal development was due to the melon genotype used as rootstock, and this effect had a significant interaction with time and FOM race. The influence of the resistant genotype is detectable also when it is used as a scion. Studies highlight differences in colonization behaviour between the two FOM races.

## Mechanism of abiotic and biotic stress tolerance in grafted vegetables

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Annual seedling production of Turkey is about 3 billion seedlings whereas more than 120 million grafted vegetable plants are planted every year. Almost 60% of the production is consisted of watermelons and followed by 30% tomatoes, 8% eggplants, 2% melons and cucumbers that are grafted before transplantation to fields or greenhouse. The choice of rootstocks determines the improvements to plant vigor and fruit yield and quality achieved by grafting. Scion resistance to biotic and abiotic stress can be enhanced by delivery from rootstocks.

Our studies are based on physiological and biochemical changes at the rootstock-scion interface in graft combinations, abiotic stress factors on *Cucurbitace* and *Solanaceae* family, and mobility of nucleic acids and proteins within grafted rootstocks.

Our forthcoming plans are consisted on the evaluation of biotic and abiotic stress resistance of scions as well as their grafting success. Thus, grafting with tomatoes is an opportunity to improve the integrity, quality and productivity of harvested fruit.

## **ROOTOPOWER: Empowering root-targeted strategies to minimize abiotic stress impacts on horticultural crops**

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The EU ROOTOPOWER project (2012-2015- grant number 289365) aims to develop new tools, targeted to the root system, to enhance agronomical stability and sustainability of dicotyledonous crops under multiple and combined stress conditions. Central to our approach is the use of tomato as a model species since it can be very easily grafted, (and indeed is usually grafted in commercial protected cropping). This surgical technique attaches genetically different shoot and root systems, allowing precise assessment of the effect of altering root traits on crop performance independently of shoot traits, since the scion (shoot) is constant. This project will analyze and exploit the natural genetic variability existing in wild-relative tomato species (used as *rootstocks*) and their beneficial interactions with natural soil microorganisms (*arbuscular mycorrhizal fungi*, *AMF* and *plant growth promoting rhizobacteria*, *PGPR*). This project will obtain genetic information and physiological understanding of mechanisms vital for high-performing root systems. The key research challenges are to (i) identify stress-resistant root systems and rhizosphere microorganisms (and their synergisms) for enhanced resistance to individual and combined abiotic stresses (ii) understand the underlying genetic and physiological mechanisms, which are potentially fundamental to all crops, and readily exploited in dicotyledonous crops. Primarily, ROOTOPOWER will evaluate rootstocks of a recombinant inbred line population from a cross between *Solanum lycopersicum* var. *cerasiforme* and *S. pimpinellifolium* for their performance under multiple abiotic stresses (drought, salinity and low nutrient supply) and for their interaction with AMF and PGPR. These genotypes represent a unique resource that has already been used to identify the first rootstock-

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specific QTL conferring salinity resistance. This project will conduct detailed analysis of the underlying rootstock-derived physiological and morphological mechanisms that influence fruit yield and quality, with special emphasis of rootstock effects on root-to-shoot signalling.

## Grafting of vegetables improves tolerance against abiotic stresses

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Due to limited availability of arable land and the high market demand, fruit vegetables are frequently cultivated under unfavourable soil and environmental conditions around the world. These conditions include drought and flooding, contamination with heavy metals and organic pollutants, nutrient deficiency, salinity, soil pH and thermal stress. These conditions cause various physiological and pathological disorders leading to severe losses in yield and quality. In our group we do research on the possibilities to overcome these damages by grafting scions onto rootstocks. Preferentially, we focus on temperature stress on tomato (*Solanum lycopersicum*) but also on other stresses. Beside screenings and selections of suitable scion/rootstock combinations for certain stresses, we are interested in the mechanisms how rootstocks change the characteristics of the whole plant and enhance its tolerance to abiotic stresses, particularly suboptimal temperatures. Here, we are using targeted and non-targeted approaches and common physiological and molecular methods for investigations.

Characteristics of interests include also tomato quality. It is determined and investigated in terms of appearance (size, shape, color, and absence of defects and decay), firmness, texture, flavour (sugars, acids and aroma volatiles) and health-related compounds (desired compounds: minerals, vitamins, and carotenoids as well as undesired compounds: heavy metals, pesticides and nitrates). Although the effect of grafting on fruit quality seems to be contradictory depending on the scion/rootstock combination and the environmental conditions, the concentration of titratable acids in tomato fruits seems to be always enhanced. Presently, we are working on the mode of action for this incident.

## Integrated strategies for control of soil-borne pathogens

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Agricultural production must be enhanced in the near future because of a growing human population and increasing competition for usable agricultural land. However, intensive crop production is usually connected with higher epidemic incidence of plant pathogens responsible for economically relevant food yield and quality losses. Hence, the agricultural production needs efficient resource and environmental friendly management strategies for disease control. Soil-borne pathogens are difficult to control even more under intensive crop production resulting in accumulation of pathogens in the soil. In the past, soil-borne pathogens were controlled mainly by the use of methyl bromide (MeBr) which was banned in 2006. Resistant cultivars and registered fungicides are often not available and alternatives are lacking. Several studies have shown that crop losses caused by soil-borne pathogens can be reduced when beneficial micro-organisms are applied. Also, grafting is most widely used in vegetable production as an effective alternative to MeBr. Cucurbitaceae and Solanaceae, such as cucumber, watermelon, and tomato, have a high importance. Both crops were affected by several soil-borne pathogens like *Fusarium* spp., *Pythium* spp., *Colletotrichum* sp. or *Rhizoctonia solani*. Hence, we propose to combine both methods, the use of beneficial micro-organisms and grafting. Through combination of both methods as a tool of Integrated Pest Management (IPM) we expect a reduction in disease severity, an enhancement of yield and crop quality and increased tolerance to abiotic stress. Because of their importance we selected two pathosystems, cucumber/*Fusarium oxysporum* and tomato/*Verticillium dahliae*, and will work on both.

## Results on grafted tomato, pepper and cucumber confirming enhanced tolerance against biotic and abiotic stresses

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Grafted plants generally showed a stronger growth and in most years a significantly higher yield compared to an ungrafted crop. The yield difference was furthermore influenced by the duration of the cultivation period and the soil health. Even in healthy soil higher yields are possible. The longer the crop was cultivated the more distinctive the yield difference. Compared among themselves the examined rootstocks yield differences were rarely significant. The increase of yield is not caused by a higher amount but by larger size of the fruits, especially in the later state of cultivation. Since the sugar content in tomatoes correlates with fruit size, in tendency it was lower in fruits of grafted plants in comparison with grafted variants. Grafting had over the years no effect on the firmness (Bareis-measured) of tomatoes.

The rootstock cultivars differed in their resistance behavior, particularly with regard to nematodes (*Meloidogyne subsp.*) and corky root (*Pyrenochaeta lycopersici*). Root evaluations at the end of the cultivation period made clear that some cultivars allow an increasing of nematodes and therefore a damaging of following crops, although the grafted tomatoes themselves showed a normal growing behaviour and high yield. Over the years especially 'Vigomax' (deRuitter) and 'Brigeor' (Enza) demonstrated a reliable resistance behavior towards *Meloidogyne ssp.*

## Effectiveness and durability of *mi*-resistant tomato cultivars and rootstocks to control root-knot nematodes

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Plant resistance is the single most important control measure that is able to suppress or retard invasion, development or reproduction of nematodes. Results from several assays conducted to know the effectiveness and durability of resistant tomato cultivars and rootstocks against the most damaging root-knot nematode species: *M. arenaria*, *M. incognita*, and *M. javanica* have shown that i) *Mi* resistant genotypes are a cost effective alternative to fumigant nematicides; ii) *M. javanica* can reproduce much more on *Mi*-resistant genotypes than *M. incognita* and *M. arenaria*; iii) homozygous (*Mi/Mi*) is more resistant than heterozygous (*Mi/mi*) ones; iv) virulent RKN populations can be selected more quickly on monocrop of resistant tomato rootstock than cultivars; v) cropping a resistant tomato cultivar for two consecutive years followed by a susceptible one provide more yield than other combinations and do not compromise resistance; vi) intermittent peaks of soil temperatures above 28°C do not affect the phenotypic expression of the *Mi* gene, either on some tomato rootstocks or on tomato cultivars.

## Grafting against abiotic stresses in vegetables crops

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In Turkey, almost all of the seedlings are produced by specialised nurseries. The number of big nurseries and transplants between 1996 and 2011 increased 33 times and %100. Although nurseries producing also grafted vegetable seedling is still limited (17), the number of grafted seedlings produced has increased tremendously. It is estimated that the number of grafted watermelon and tomato seedlings is over 100 million. Also grafted cucumber, melon and eggplant seedlings have been produced. In Turkey, first researches on vegetable grafting have started in 1980s. However commercial use has increased in 2000s.

Our group has conducted many researches during last ten years. Response of different rootstocks against abiotic stresses (i.e. salinity, water stress) was tested. The crops were tomato (~ 65%), cucumber, melon, watermelon and eggplant. The screening tests in those crops were conducted in water culture. Mycorrhiza was used in some experiments in order to reduce the negative effect of salinity stress.

A new project will be conducted aiming to determine tolerance/resistance of eggplant genotypes provided from different resources and including different species against biotic (i.e. Fusarium and Verticillium wilts, nematode) and abiotic (i.e. low temperature and salinity) stress factors. The results obtained within the framework of the project will be shared with the private sector since it is targeted to use by the seedling nurseries.

In this presentation, a brief information will be given related to some of our results from our experiments and future plans.

## Grafted watermelon, melon and cucumber in isarel: agro-technology used for reducing soil-borne disease damage

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Grafting cucurbits have been used in Israel for 15 years. Grafting expanded following the ban on methyl bromide for soil disinfestation.

**Watermelon**- watermelons are grafted onto *Cucurbita* rootstocks to overcome the vine-decline phenomenon caused mainly by *Macrophomina phaseolina*, alone or combined with other soil-borne pathogens. Grafted watermelon transplants are placed two meters apart in the row rather than one meter apart as in non-grafted watermelons. This change allows some reduction in plant investment without reduction in yield. Fruits from grafted plants often exhibit reduced quality. The research goal was to look for optimal rootstock-scion combinations and agro-technology to avoid this drawback. For the long term, there is a need to learn the physiology of compatibility and to develop new watermelon rootstocks, mainly for mini-watermelons.

**Melon**–Galia type melons have been grafted onto *Cucurbita* rootstocks for overcoming wilting caused by *Monosporascus cannonballus* in the 'Arava Valley of southern Israel. Ananas-type melons grown for the local market in northern Israel are also grafted to reduce damage caused by *Macrophomina phaseolina*.

**Cucumber**–Grafted cucumbers are used mainly to avoid damage caused by Fusarium crown rot caused by *Fusarium oxysporum* f. sp. *radices cucumerinum*. Grafting leads also to vigorous growth, especially in winter, contributing to higher yields and improved performance against nematodes and soil infection with *Cucumber green mottle mosaic virus* (CGMMV).

## Response of grafted tomato to boron excess

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Boron toxicity represents one of the most feared abiotic stresses limiting vegetable production in certain coastal areas of the Mediterranean Basin. In Apulia (Southern Italy), the boron concentration of irrigation water in some cases exceeds 4 mg/L. In the same region and in Sardinia, there is a growing interest for the use of beached posidonia (*Posidonia oceanica* (L.) Del.) residues as soilless growing media component or as composting material; however, their use in agriculture may be limited by the high boron concentration that characterizes this seaweed. To face these issues, the research group of the Experimental Farm 'La Noria' is conducting several research activities aimed to investigate the potential role of vegetable grafting in improving tomato crop tolerance to boron excess within the research project titled "Physiological response, growth, yield, and quality of grafted tomato under combined excess boron and salinity stress", and funded by the Italian Ministry of Education, University and Research (MIUR, PRIN 2009). Moreover, other research activities are in progress within the project LIFE09 ENV/IT/000061 "Posidonia Residues Integrated Management for Eco-sustainability" to evaluate the effectiveness of tomato rootstocks in alleviating the boron phytotoxicity in tomato plants grown on posidonia-based compost characterized by high boron content.

## Improvement of abiotic stress tolerance through the use of vegetable grafting

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In view of exploring the unveiled potential of vegetable grafting technology, the research activities in this area at the Department DAFNE, Tuscia University, Viterbo started in 1997 with special emphasis on improvement of abiotic stress tolerance in cucurbits (melon, watermelon, and cucumber). Many of the research activities have been done in collaboration with other Italian and International research groups. At the beginning, the abiotic stresses taken into consideration in vegetable grafting researches were salinity, drought and alkalinity. The major activities have been going on here covers the screening and selection of tolerant rootstocks and revealing the mechanisms involved in grafted plants tolerance, including agronomical, and physiological aspects against these stresses. Recently, a research project has been conducted with the aim to improve nitrogen use efficiency in watermelon and melon through the selection of suitable *Cucurbita* rootstocks. In several experimental trials, interactions of scion and rootstock have been also considered in terms of fruit quality with particular emphasis on nutraceutical value of the product (eg. antioxidant compounds). The researches on integrated approach by involving suitable AM fungi for increasing performance of grafted plants against abiotic stresses have also been considered. Our present research aims at alleviating metals toxicity, including heavy metals in cucurbitaceous and solanaceous crops. Our aim is to alleviate plant tolerance to Al toxicity in cucumber while Cd and Ni toxicity in tomato, besides to reduce the heavy metals (Cd and Ni) concentrations in the edible fruits of tomato. We are also trying to find suitable and compatible AM fungi and bacterial strains for further improvement of plant tolerance against such stresses.

## Soil solarization in combination with grafting for the control soil borne plant pathogens

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Soil solarization is a nonchemical soil disinfestation method able of exploiting solar energy for heating soil mulched with a transparent PE or impermeable VIF sheet, and reaching soil temperature up to 40-55°C in the upper soil layer. Solarization is able to control effectively soilborne pathogens and other soilborne pests such as nematodes and weeds. The major constraints that limit the adoption of soil solarization in practice are the relatively long duration of the process, land availability and the climatic dependency. Plant grafting is also widely used for controlling soilborne pathogens and pests. The integrated use of solarization with grafting appears to be a sustainable alternative for excluding or restricting the use of soil fumigants. Most importantly, this combination had proven able to secure control of a great variety of soil borne pathogens beyond those controlled by the resistant rootstocks and achieving an additive effect on yield. Mechanical or manual applications of soil solarization for the control of soil borne pathogens in plastic houses or in field crops in Greece, demonstrated that solarization is effective in controlling *Fusarium* and *Verticillium* wilts of watermelon. The use of *Fusarium* resistant rootstocks in watermelon cultivations protects the plants from *Fusarium oxysporum f.sp. niveum* but not from *Verticillium dahliae*. The cost of machine application of soil solarization in the Amaliada region of Greece is very low (750 Euro per ha) compared to the 6-8 times higher cost of fumigants. Similarly, soil solarization in Cyprus is highly effective against *Verticillium* wilt but only partially effective against corky root rot (*Pyrenochaeta lycopersici*) and root-knot nematodes (*Meloidogyne* spp.); but controls adequately most annual weeds. By contrast, grafting provides complete protection from corky root rot and root-knot, but only partial protection against *Verticillium* wilt. Solarization or grafting provides significant yield increases over that of the untreated control. However, a combination of both is much more effective. The integrated use of solarization and grafting appears to be a sustainable alternative to methyl bromide fumigation.

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## Effect of grafting on plant response to abiotic stress factors in cucurbits

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The collaborating departments at Corvinus University of Budapest are interested in the use of grafting for protection of melon, watermelon and cucumber cultivars against abiotic stress factors, in revealing the physiological and molecular processes behind this phenomenon and in understanding graft compatibility.

In Athens we would like to present results on evaluation of salt tolerance of watermelon (cv. Esmeralda F1) grafted on *Lagenaria* (L, DG-01 F1) and *Cucurbita maxima* x *Cucurbita moschata* interspecific rootstock (IS, Shintosa F-90 F1).

Plants were grown in controlled environment (150/0  $\mu\text{mol s}^{-1} \text{m}^{-2}$ , 25/20°C, 70/70% RH). Plants with three fully expanded leaves were watered with mineral nutrient solution containing 0 and 120 mM NaCl. Physiological parameters were determined after 21 days of treatment.

Salt treatment decreased root and stem water content of non-grafted and self-grafted watermelon, while that of plants grafted on L and IS did not show significant decrease. The treatment also decreased transpiration and net photosynthesis through lowering of stomatal conductance, but IS-grafted plants maintained a relatively high net photosynthesis. Significant growth inhibition by salt treatment was only observed in foliar growth of non-grafted plants, while root, stem and foliar growth of all grafted plants was maintained, IS-grafted plants had a remarkable growth rate.

## Impact of grafting and rootstock genotype on mineral uptake by fruit vegetables

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Several studies have indicated that the uptake and/or utilization efficiency of macronutrients by plants may be enhanced by grafting onto some rootstocks. This is ascribed mainly to the root characteristics of these rootstocks, which are more vigorous than those of highly productive cultivated varieties. However, other mechanisms implicated in the efficiency of active nutrient absorption by the roots, as well as signals arising from the scion, which are mainly governed by sink demand, may also enhance element uptake and utilization. On the other hand some rootstocks are capable of restricting the uptake and/or the transport of macro- and micronutrients to the shoot, thereby mitigating stress caused by excessive external concentrations of element such as Ca, Cu, B, Cd or Ni. Although the rootstock genotype determines the uptake rate of an element, the responses of the grafted plant to an excessive concentration of this element is ultimately determined by the scion. In the current short presentation, a survey of recent investigations carried out at the Laboratory of Vegetable Crops of the Agricultural University of Athens, which were aimed at evaluating the efficiency of some rootstock/scion combinations of fruit vegetables to take up and utilize nutrients and ameliorate nutrient or heavy metal toxicities will be presented. These include the impact of some commercial tomato rootstocks on mineral nutrition, salt tolerance and micronutrient deficiencies or toxicities in grafted tomato, the responses of cucumber to heavy metal stress owing to excess Cd, Ni, or Pb levels in the root zone, and the impact of mycorrhiza and other symbiotic microorganisms on uptake of nutrients by grafted tomato.

## Emerging pest and diseases on grafted plants

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The lack of registration of soil fumigants including pre-plant nematicides and fungicides represents a technical limitation to be faced particularly for intensive protected Solanaceae and Cucurbitaceae grown in Italy. Actually grafting technique is a feasible technique for several growing areas in Italy. Since 2009 the effectiveness of grafting was evaluated under different circumstances, always representing standard growing condition for protected tomatoes, eggplants and melons. On the basis of the evaluation of galling index, experimental trials did not show significant effects of rootstock against *M. javanica*, *M. arenaria* and *M. incognita*. On *Solanum torvum*, widely adopted as eggplant rootstock, *Verticillium dahliae* was identified as vascular disease. On tomato rootstocks *Colletotrichum coccodes*, *Rhizoctonia* sp. and *Phytophthora* sp. were identified as causal agents of basal and root rots. Particularly on melon and water melon rootstocks infections of *Macrophomina phaseolina* and *Rhizoctonia* sp. were often identified. On the basis of this observation grafting technique cannot be considered as a unique solution to manage soilborne pest and disease. Nevertheless grafting on resistant rootstocks represents an essential tool in a more complex strategy able to make possible the cultivation of Solanaceae and Cucurbitaceae crops under intensive growing systems.

## *Section 5*

### *WG4*

## *Rootstock-mediated improvement of fruit quality*

## **Contribution to the COST Action FA1204: Vegetable Grafting to Improve Yield and Fruit Quality under Biotic and Abiotic Stress Conditions**

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In the Netherlands, over 90% of the tomato and egg-plants cultures consist of grafted plants. In important crops as pepper and cucumber, rootstocks are still hardly/not used, however, the search for rootstocks that can improve the cultivation of these crops continue. The economic importance of grafting vegetable crops in The Netherlands and Europe is large, for its opportunities to improve crop production levels, as well as for the possibilities rootstock can offer in dealing with soil diseases, nutrient shortage, salinity and drought (especially in South/East Europe).

Over the last years, research at Wageningen UR Greenhouse Horticulture has been conducted to make an inventory of rootstocks and their characteristics that can be beneficial in energy-efficient cropping systems and to look for rootstocks suitable for biological cropping systems of fruit vegetables. Recently, app. 150 tomato rootstock genotypes have been grown at Wageningen UR Greenhouse Horticulture to test their performance under abiotic stress (nitrogen shortage) as part of the EU project ROOTOPOWER. Furthermore, in the EU project PLANTPOWER, root exudation of different tomato rootstock genotypes was examined.

In this COST Action, Wageningen UR will primarily contribute to the work packages concerning rootstock-scion interactions and the rootstock-mediated resistance to biotic and abiotic stresses.

## Improving salt tolerance

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Salinity is an abiotic stress factor variable in time and space. It affects yield quality and quantity, and almost every aspect of the physiology and biochemistry of the plant. Wild tomato species, like *S. cheesmaniae* or *S. pimpinellifolium*, have often been considered useful source of salt tolerance genes to transfer to cultivated tomato. Advantageous alleles at all fruit weight QTLs have been found to come from the cultivated, salt sensitive, species, while reduced expression of the wild allele of HKT1;2 in roots appeared related to more Na<sup>+</sup> transport to the aerial plant.

Grafting is an old-known biotechnology tool to improve the amount and uniformity of crop yield. The rootstock effect on fruit yield of a grafted tomato variety under high salinity was found heritable ( $H^2$  near 0.3) and governed by at least 8 QTLs. Since the advantageous allele generally came from the wild, salt-tolerant species, it was concluded that a more efficient utilization of wild germplasm would be via the improvement of the rootstocks that confer salt tolerance. Correlation and QTL analysis suggests that rootstock-mediated improvement of fruit yield in tomato and *Citrus* under salinity is mainly explained by the rootstock's ability to minimise perturbations in scion water status.

## Influence of grafting on fruit vegetables quality

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Since the 90's our work has been focused in the behaviour of grafted plants normally used against soil-borne diseases and how yields and fruit quality are affected.

We firstly work in watermelon grafted plants, concluding the benefits of this technique in terms of yield, selecting the best rootstocks and quantifying the quality of fruits from grafted plants. The yields of grafted plants using *C. maxima* x *C. moschata* were higher and had no effect on the soluble solids concentration. Not colour or internal breakdowns were affected.

In melon, our studies on the grafting technique have focussed in founding the most compatible rootstocks and how also yields and quality are affected. Fruits from grafted plants have been found to have higher soluble solids content and higher in colour parameters (yellowness). The technique of double grafting has also been evaluated to avoid affinity problems in the Spanish melon cultivars.

Now we are focussed in sweet pepper plants cultivated in water and salt stress conditions, searching for adequate rootstocks and evaluating the tolerant rootstocks to these stresses in terms of yield and quality both in stressing and normal soil conditions. Fruits from the selected tolerant rootstocks have lower percentages of blossom-end-rot without affecting fruit size.

## Impact of grafting of tomato plants on fruit quality varies according to quality parameter

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Grafting plants on a rootstock has become common practice in year-round cultures of tomato on artificial substrate, for trials and practical experience show that grafting on a vigorous rootstock increases production, especially in the autumn period. Grafting does not have a serious impact on fruit quality. However, research has shown that fruit symptoms of Pepino Mosaic Virus in tomatoes can be decreased by grafting. In two types of loose tomatoes less open and damaged fruits were seen. There was also a tendency toward decreased fruit marbling in the grafted plants. Moreover, the green parts of clusters of grafted plants conserve better and their fruits show less microcracks. On the other hand, tomatoes of non-grafted plants tend to be less ribbed and less angular-shaped. Also, firmness after conservation tends to be better in fruits of non-grafted plants. Fruit shape in grafted plants can be improved by creating two-headed tomato plants. Making two-headed plants by keeping an extra stem in an early stage, on the level of the cotyledons, can improve fruit shape even more. On the other hand, clusters of grafted plants with one head have courser green parts.

## Quality of agricultural production as influenced by grafting

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In recent decades there has been a growing demand of better nutritional, nutraceutical composition, and better tasting fresh vegetables. The levels of nutrients and antioxidants are affected by genotype, maturity and growing conditions. Grafting is an important technique for dealing with soilborne pathogens and abiotic stresses. Also grafting practices could influence plant growth, yield, and quality of scion fruits. Rootstock-scion interactions and modification of the nutrient value of fruits from grafted plants are important to optimize the yields based on the amount of nutrient production per unit area, resulting in a more sustainable agriculture which supplies consumers with nutritionally balanced food. Our studies are focused on assessing the effects of grafting on yield, the dry matter content, total soluble solids, fruit mineral composition (potassium, phosphorus, calcium, magnesium, sodium and minor minerals such as iron and zinc), proteins, and antioxidant compounds as phenolic substances, carotenoids and the ascorbic acid content of grafted and ungrafted pepper and eggplants.

## Improving grafting techniques and fruit quality assessment on eggplant and watermelon grafted plants

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Grafting has become an important cultural practice for vegetable crop production in Sicily. This has resulted in increased efforts to study specific aspects of grafting. Our previous projects have focused on two research lines. The first was aimed at studying the influence of grafting on eggplant fruit quality. Results showed that the concentration of Na, Mg, Mn in fruits from several Sicilian eggplant ecotypes grafted onto *Solanum torvum* seedlings were lower than those from non-grafted plants. Grafting onto *Solanum torvum* resulted in higher flavonoid content. This in turn has favourable effects on human hypertension and blood pressure. The second research focused on the use of un-rooted triploid watermelon grafted plants to optimize healing time condition and shipping. Valuable information were available on the healing period requirement and seedling quality. Future research activities will focus on the possibility to reduce the propagation time required to produce grafted eggplants. The method involves the use of unrooted eggplant grafted cuttings (eggplant cultivar scion and *Solanum torvum* rootstock). This propagation system would allow more flexibility on the availability of grafted plants, transportation at low temperature of grafted plants without roots, and resolve the problem associated with the low percentage *Solanum torvum* seed germination.

## Quality and postharvest performance of watermelon in response to grafting on cucurbit rootstocks

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Our work focuses on the effect of rootstocks on watermelon scion fruit quality and postharvest performance. Yield characteristics are examined in conjunction to fruit quality. Particular emphasis is placed on *Cucurbita maxima* × *C. moschata* and *Lagenaria siceraria* rootstocks. Scion cultivar assessment encompasses diploid and triploid, large and small-fruited commercial cultivars as well as local heirloom varieties revisited. Fruit quality in response to grafting is assessed during fruit ripening, at the time of harvest and during postharvest storage. Fruit quality is assessed in terms of morphological, physicochemical, nutritional, and bioactive components. Physicochemical characteristics include flesh firmness, flesh color, soluble solids content, pH and titratable acidity of the fruit juice. Nutritional and bioactive components include soluble carbohydrates (glucose, fructose, sucrose), lycopene, citrulline, meloxicam and free-radical scavenging capacity. Inter-specific hybrid rootstocks improved yields consistently across seasons and cultivars, while no rootstock examined resulted in severe reduction of fruit quality. On the contrary, flesh firmness was consistently improved by all hybrid rootstocks tested. Hybrid rootstocks further improved postharvest lycopene content and enhanced the colour intensity of the flesh.

## Optimizing vitamin E and K production by grafting of tomato crop varieties

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Within the framework of the COST action, we will present a proposition for a collaborative research project between the research groups of Camps (Agroscope) and Kessler (University of Neuchâtel, Switzerland).

Agroscope is an experimental research station where trials on tomato and cucumber "soiless" cultivation in greenhouse are performed and perfected. Main topics during the last years were: effects of greenhouse climate management on tomato quality taking into account the varieties and rootstock.

The Kessler laboratory in Neuchâtel is specialized in metabolic analysis of plants under abiotic stresses and development and application of innovative phytochemical analytical methods. In particular, the laboratory together with the chemical analytical service (CAS) at the Neuchâtel acquired and uses state-of-the-art expertise in prenylquinone (eg Vitamin E, Vitamin K) and carotenoid (eg carotenes, lycopene) profiling as well as other metabolomic analyses. Initially developed in the Arabidopsis model system, this knowledge can readily be applied to tomato fruit and as well as other crop plants.

Together the teams will develop collaborative research aimed at understanding and optimizing the effects of tomato grafting on accumulation of carotenoids and prenylquinones in tomatoes. Initially, we will establish a comprehensive method to rapidly determine the carotenoid profile using UHPLC-QTOFMS analyses. The knowledge and developed methodology will be applied to analyze grafted tomato plants (graft varieties: Merlice (DRW7812) (DR), endeavour (RZ), Levanzo (RZ) and rootstock varieties: Maxifort (DR), Beaufort (DR), Emperador (RZ), Kaiser-77 (RZ)) under abiotic and/or biotic stresses. Modified traits will be identified by screening tilling collections in the M82 variety available from Kessler's collaborator Dr. Bendahmane in Evry (France).

## Effects on yield and quality of grafted tomatoes with two pruning systems and organically amended with different composts

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This study aimed to evaluate the effects on tomato yield and quality, of two pruning systems of grafted tomato plants (cv. Valoásis M40 F1 and Maxiforte) used to prevent the incidence of soil diseases and of the soil application of different composts. The trial was conducted with spring/summer crops in a greenhouse (NW Portugal), with a three block split-plot experimental design. The large plots included treatments without compost and with three composts of the source-separated organic fraction of municipal solid wastes (conventional, conventional with pine bark and certified for organic production) ( $7.3 \text{ t ha}^{-1}$ ). The small plots included 2 pruning systems of grafted tomato: double and triple stem.

The total yield was not significantly different between crops grown without and with the soil application of the three composts, probably due to the high soil fertility, but fruit quality was improved with compost application, producing firmer and less acid fruits, compared to fruits grown without compost. The fruit grade  $> 102 \text{ mm}$  represented about 76% of total yield and, for this grade, the double-stem tomato plants increased yield ( $14.5 \text{ kg m}^{-2}$ ), compared to plants with three stems ( $13.5 \text{ kg m}^{-2}$ ), which offset the increased cost of the number of required plants.

## Vegetable grafting to improve yield and fruit quality under biotic stress conditions at the COMAV institute

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Grafting of vegetable crops is one of the major research lines at the COMAV Institute (Universitat Politècnica de València, Spain). In order to use this technique to improve yield and fruit quality under biotic stress conditions, different works have been carried out to develop rootstocks able to cope with several pathogens that limit pepper, eggplant, and melon production. Thus, we have studied the aggressiveness and genetic diversity of *Phytophthora capsici* isolates infecting pepper and has evaluated germplasm for *P. capsici* and nematode resistance. This allowed the development of two hybrids which showed good levels of resistance and agronomic performance when used as rootstocks. Also, we have selected germplasm that may carry different *Meloidogyne incognita* resistant genes. The performance of eggplant grafted onto cultivated, wild, and hybrid materials of eggplant and tomato was also evaluated in nematode infected yields. The results suggest that *S. melongena* germplasm and both intraspecific and interspecific eggplant hybrids (*S. melongena* × *S. aethiopicum*) seem to be promising materials for developing new rootstocks for eggplant production. We have set up procedures for screening melon germplasm against *Monoporascus cannonballus*, the causal agent of melon vine decline, including, inoculation, root analysis and, qPCR quantification of the pathogen. Resistance to this fungus has been found in exotic sources and introgressed into different genetic backgrounds. Grafting melons onto melon lines with improved roots, resistant to soilborne pathogens, is a promising method to reduce the impact of grafting on melon quality.

## Vegetable grafting to improve yield and fruit quality under different Greek environmental conditions

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Since 2000, in both laboratories of Vegetable Production and Plant Genetic and Breeding in the University of Thessaly in Greece, several research works have been carried out regarding the description of Cost topic. In particular, the research works on the effects of grafting on growth performance and yield under different environmental conditions of aubergine, tomato, melon and watermelon. The results of these mentioned works showed that seedling of grafted plants had significantly more fruits than un-grafted plants. Also, research works were studied in relation to fruit storage time (up to 17 days at 10°C) and the physicochemical parameters and sensory parameters of grafted and un-grafted. The fruits were stored under modified atmosphere packaging (MAP) and found that Flesh firmness was negatively affected by grafting and reduced over storage, but positively affected by MAP on melon and on aubergine. Moreover, in another experiment on examining the effect of grafting on aubergine leaf photosynthetic gas exchange, it was found that the rootstock seems to be able to modify the scion leaf photosynthetic capacity depending on scion/rootstock combinations. Also, in evaluating rootstock for watermelon grafting at two growing temperatures 8<sup>o</sup> C and 16<sup>o</sup> C with reference to plant development, was found that yield and plant quality of grafting watermelon results were better in plant growth by the time of transplanting. However, the scion-rootstock combination affects on fruit quality and at minimum temperature of 16<sup>o</sup> C was indicated during the post-grafting stage. Furthermore, in an experiment with fifty two Greek tomato cultivars, accessions and ten commercial nematode resistant tomato cultivars as well as different rootstocks were evaluated under control environmental conditions for resistance against the root-knot nematode *Meloidogyne joavanica*. The result showed that all tested local tomato cultivars and accessions were susceptible to *M joavanica* except the commercial resistance cultivars and some rootstocks showed a different response.

We think that the COST Action Programme aims to develop a multidisciplinary network of partners and will give a major opportunity to understand the mechanisms of rootstock-mediated crop improvement for fruit quality, productivity and sustainability under

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multiple and combined stress factors. Sharing knowledge, scientific and technical collaboration will surely enlarge the knowledge in the area of vegetable grafting. In this regards in both above mentioned laboratories could extent further their research works towards the objective of the COST programme.

## Rootstock for organic fruity vegetables

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In heated organic greenhouse production, companies focus on fruity vegetables in order to justify investments and technical features. This leads to limitations in crop rotation. Eggplant, cucumber and pepper are obvious as rotating crops in alternation with tomato. Unfortunately, this types of vegetables are host for the same kind or soil-borne diseases and nematodes. Growers are forced to use grafting. Rootstocks should combine resistance with good compatibility between rootstock and graft, good yield and quality. In 2009 and 2010 the organic division of the Vegetable Research Centre Kruishoutem performed trials on rootstock. In 2009 the sweet pepper variety Ferrari (Enza) was grafted on Capital (De Ruiter) in a single stalk cultivation method in the organic greenhouse of the PCG and compared to identical non-grafted reference in 5 parallels in a randomized block design. Grafting had no advantage in this single stalk concept. In 2010 two trials on location (Belgium and the Netherlands) for grafting eggplant were tested. The results were depending on the soil type, the compatibility, .... On average, combination of the variety Taurus (Monsanto) on rootstock MAO (Eminent Seeds) had the highest production level, the healthiest crop and roots. In the beginning the growth speed was slower. During the culture, the production ran out the other combinations. In the summer of 2010 rootstock trials were performed in the cucumber-variety Amazone (Monsanto) in a randomised block design in 3 parallels. Each rootstock had his own properties. Bombo (Monsanto) enhanced the production level, Harry (Syngenta) had positive influence on powdery mildew inhibition, RS 3518 and RS3535 (Uniseeds) produced glossy fruits. Rootpower (Sakata) was on average a good rootstock.

## *Section 6*

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#### *Closing*

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