SAPIENZA

Università di Roma

Facoltà di Scienze Matematiche Fisiche e Naturali

DOTTORATO DI RICERCA
IN BIOLOGIA CELLULARE E DELLO SVILUPPO

XXVII Ciclo
(A.A. 2013/2014)

Physiological and molecular aspects of the melon-*Fusarium oxysporum* f. sp. *melonis* race 1.2 pathosystem

Maria Silvia Sebastiani

Docente guida

Prof. Giulia De Lorenzo

Tutore

Dr. Nadia Ficcadenti

Coordinatore

Prof. Rosa Sorrentino

Index

1. SUMMARY

Fusarium oxysporum f.sp. *melonis* race 1.2 (FOM1.2) is the most virulent and yield-limiting pathogen of melon (Cucumis melo L.) cultivation worldwide. Colonization of plants by FOM leads to necrosis of the infected tissues, collapse of vascular vessels and decay of the plant. Resistance to FOM1.2 appears to be controlled by multiple recessive genes and strongly affected by environment. A RNA-Sequencing approach was used to investigate the transcriptome dynamic during incompatible and compatible interactions for the identification of candidate resistance genes in the melon-FOM1.2 pathosystem. The doubled-haploid (DH) resistant line NAD and the susceptible cultivar Charentais-T (CHT), both inoculated with FOM1.2, were analyzed at 24 and 48 hours post-inoculation (hpi). The landscape of differentially expressed genes (DEGs) diverged significantly in the two genotypes. Transcriptome analysis of NAD identified 2461 and 821 DEGs (82% up-regulated at 24 hpi and 69% at 48 hpi), while in CHT 882 and 2237 DEGs were recovered at 24 hpi and at 48 hpi, respectively (29% up-regulated at 24 hpi and 81% at 48 hpi). Several unannotated transcripts were found to be modulated, providing a basis for further exploration of plant defense-related genes. NAD, unlike CHT, modulates a higher proportion of up-regulated genes at 24 hpi, suggesting a more prompt response. Gene ontology (GO) enrichment of DEGs highlighted that ″defence response, incompatible interaction″ and ″response to stress″ GO groups emerged as major effectors of NAD resistance to FOM1.2. Both constitutive and inducible defense responses contribute to reduced FOM1.2 vascular colonization of melon resistant genotype. Of particular interest were transcripts involved in the cell wall reinforcement and disease resistance genes including *FMO*1, *E3 Ubiquitin protein ligase* and *pathogenesis-related thaumatin encoding genes*. Although the early expression of *ankyrin-repeat containing genes*, probably related to salicylic acid (SA) pathway, in NAD FOM1.2 resistance seems mainly mediated by the crosstalk among jasmonate/ethylene (JA/ET), auxin and abscissic acid (ABA) hormone signaling networks. Fungal transcripts expressed *in planta* were also detected for identifying potential virulence effectors. The achievement of candidate resistance genes and the identification of unique melon sequences with unknown functions required the establishment of efficient, genotype-specific melon regeneration and transformation protocols for future functional genomics studies. Three cultivars (CHT, Vedrantais and Isabelle) and three DH melon lines (NAD, DH-L2 and DH-L6) were tested for their potential to regenerate under *in vitro* conditions. The results confirmed that the hormonal requirement and the genotype strongly influence plant regeneration in melon. In order to confirm genetic stability of the regenerated melon plants a PCR-based Random Amplified Polymorphic DNA (RAPD) analysis was carried out using 20 decamer-primers.

2. INTRODUCTION

2.1 The molecular basis of plant pathogen interactions

In their natural environments, plants are under continuous biotic stress caused by different attackers (e.g., bacteria, fungi, viruses and insects) that compromise plant survival and offspring. Plants have developed molecular mechanisms to detect pathogens and pests and have evolved a variety of resistance mechanisms that can be constitutively expressed or induced after pathogen or pest attack. Plants lack a somatic adaptive immune system and therefore mobile defender cells and rely on an innate immune system that is based on the specific detection by plant pattern recognition receptors (PRRs) of relatively conserved molecules (elicitors) of the pathogen called pathogen-associated molecular patterns (PAMPs). This type of resistance response is known as PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). The elicitors may be glycoproteins, peptides, lypopolysaccharides on the cell surface of Gram-negative bacteria (LPS), fungal chitin fragment, N-mannosylated glycopeptides, ergosterol, flagellin and harpins (Nurnberger et al., 2002). Successful pathogens secrete effector proteins that deregulate PTI. To counteract this, plant resistance (R) proteins recognize effectors and activate effector-triggered immunity (ETI) (Dodds and Rathjen, 2010). A finely tuned regulation of these immune responses is necessary because the use of metabolites in plant resistance may be detrimental to other physiological processes impacting negatively in other plant traits, such as biomass and seed production (Walters and Heil, 2007; Kempel et al., 2011). The current view of the plant immune system can be summarized in a 4 step zig-zag-model (Figure 1; Jones and Dangl, 2006).

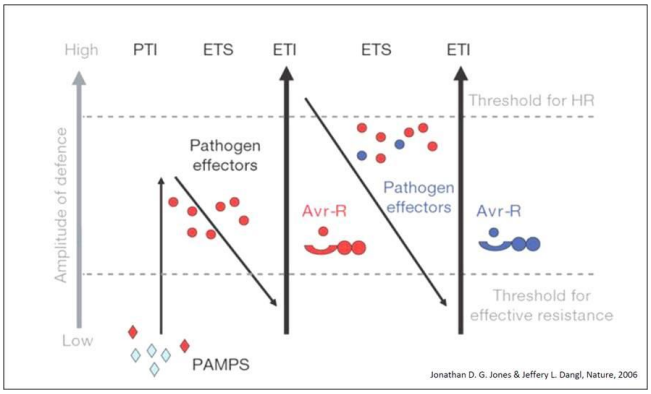


Figure 1. A zigzag model illustrates the quantitative output of the plant immune system.

PAMPs are directly recognized by PRRs. This leads to PTI and the infection is halted. One reason that not all pathogens are successful on all plants is the so called non-host resistance. This means that there is only a limited number of possible host available per pathogen. For the rest is either the effector ineffective or can be immediately recognized. Only successful pathogens survive PTI which means that they need to have effectors which can interfere with PTI. This results in effector-triggered susceptibility (ETS). The used effector is recognized by a specific disease resistance gene. The effect resulting from this is ETI and is basically an accelerated and amplified PTI response which results in disease resistance and usually hypersensitive cell death response (HR). Hence, the timely recognition of an invading microorganism coupled with the rapid and effective induction of defense responses appears to make a key difference between resistance and susceptibility. In nature, the dynamic relations between plants and their pathogens are under control by a co-evolutionary relationship, a process whereby the host and the pathogen species contribute reciprocally to the forces of natural selection they exert on each other (Staskawicz, 2001). Plant pathogens can be broadly divided into those that kill the host and feed on the contents (necrotrophs) and those that require a living host to complete their life cycle (biotrophos or hemibiotrophic). Necrotrophs produce toxins and/or enzymes and plant resistance can be achieved via the loss or alteration of the toxin's target or through detoxification. The biotrophos or hemibiotrophic are recognized by the plant innate immune system that is highly polymorphic in its capacity to recognize and respond to them (Hammond-Kosack and Jones, 1997; Dangl and Jones, 2001). Except for the RNA-based antiviral defense which is a form of adaptive immunity, the plant defense mechanism is comparable to an innate immune system, similar as the human one (Jones and Takemoto, 2004; Nurnberger et al., 2004). The signaling divides here between biotroph and necrotroph organisms. Many pathogens establish their first contact with plant cells in the apoplast, the extracellular space in plant tissue that constitutes a source of nutrients and shelter for many microbial inhabitants. At the same time, the apoplast is a hostile environment that contains hydrolytic enzymes and toxins that may challenge microbial growth. Furthermore, host hydrolytic activities establish decomposition of microbial matrices to generate soluble PRR ligands (Liu et al., 2014). For instance, apoplastic glucanases and chitinases disrupt the integrity of fungal walls and release chitin and glucan microbe associated molecular patterns (MAMPs). In response, several strategies evolved in plant pathogens in order to prevent recognition and MAMP-triggered activation of immune responses, including alterations in the composition and structure of cell walls, modification of carbohydrate chains and secretion of effectors to provide protection to the cell wall or target host immune responses. In pathogenic fungi, various types of effectors have been characterized that either protect their cell walls or prevent or perturb the elicitation of cell wall-triggered host immune responses (Kombrink et al., 2011; Rovenich et al., 2014). Especially the role of chitin in interactions of pathogenic fungi and host plants has received considerable attention, as it has been known for decades that plant hosts respond to chitin application by inducing immune responses. In pathogenic fungi, the cell wall plays an important role during host invasion. It is the first structure of the pathogen to make physical contact with host cells, which may recognize several of its components as MAMPs in order to activate host immune responses (Thomma et al., 2011; Latge and Beauvais, 2014). Like other organisms, also plants evolved to recognize MAMPs by cell surface localized PRRs to mount an immune response. The best-studied plant PRRs are the sensors for bacterial flagellin (FLS2) and elongation factor Tu (EFR), respectively (Gomez-Gòmez et al., 2001; Zipfel et al., 2006). These are transmembrane proteins that carry extracellular leucine-rich repeats (LRRs) and a cytoplasmic kinase domain. However, although LRR-type PRRs are the most studied, PRRs may also carry other extracellular domains than LRRs to perceive microbial ligands (Antolìn-Llovera et al., 2012). For instance, plant receptors for fungal chitin and bacterial peptidoglycan contain extracellular lysin motifs (LysMs) that were initially discovered in peptidoglycan-hydrolyzing enzymes (Wan et al., 2008; Willmann et al.,

2011). The first chitin immune receptor gene that was cloned was the gene encoding the rice chitin elicitor-binding protein (CEBP), a cell surface localized receptor with extracellular LysMs that lacks a cytoplasmic signaling domain such as a kinase (Kaku et al., 2006). CEBP is essential for chitin recognition and indispensable for the induction of chitin-triggered immunity (Kaku et al., 2006). The plant kingdom contains thousands of R-genes with specificities for particular viral, bacterial, fungal, or nematode pathogens. Although there are differences in the defense responses induced during different plant-pathogen interactions, some common themes are apparent among R gene-mediated defenses. Most significantly, the function of a given R gene is dependent on the genotype of the pathogen (Bent, 1996). The homology between resistance proteins led to a suggestion that they may function in a conserved pathway in eukaryotes, which is activated in response to pathogen challenge (Lehmann, 2002). Striking similarities are found in the structures of R proteins from monocotyledonous and dicotyledonous species, implying that fundamental modes of recognition and defense signaling have been retained through plant evolution and diversification. There is a high similarity between some R proteins and the antifungal polygalacturonase-inhibitor proteins (PGIPs) that belong to the superfamily of LRR proteins. PGIPs are glycoproteins located in plant cell walls that specifically inhibit fungal endo-polygalacturonases (PGs) that are the first cell wall-degrading protein to be secreted by fungal pathogens (Bent, 1996; De Lorenzo et al., 1999; Di et al., 2006; Ferrari et al., 2012). One function of R-mediated signaling is to more rapidly and effectively activate a cascade of defense mechanisms that are shared by both basal and induced resistance.

2.2 Plant defense signal transduction pathway

Plants possess constitutive as well as inducible defense systems to detect extra and intracellular pathogen-derived proteins that function as eliciting stimuli to initiate the plant's primary defense response. The recognition mechanism, shared between specific and basal resistance R proteins-mediated, initiates a series of signaling cascades that coordinate the initial plant response to impair pathogen ingress, leading to disease resistance (Veronese et al., 2003). Diverse small-molecule hormones play pivotal roles in the regulation of this network. Their signaling pathways cross-communicate in an antagonistic or synergistic manner, providing the plant with a powerful capacity to finely regulate its immune response. Pathogens, on the other hand, can manipulate the plant's defense signaling network for their own benefit by affecting phytohormone homeostasis to antagonize the host immune response. Despite the diversity of pathogens, plants relay on many of the same signal transduction components to bring about cellular changes that limit the growth and spread of invading organisms. The downstream cellular events that mainly characterize the resistant state are:

- Cell wall strengthening by means of lignin and callose deposition and papillae formation, acting as first line of defense that plant pathogenic fungi encounter to colonize the plant tissue and obtain nutritional requirements.
- Alteration of membrane potentials.
- Rapid oxidative bursts leading to production of reactive oxygen species (ROS) and nitric oxide (NO) that are considered key signals in plant defense.
- Transcriptional reprogramming that leads to several defense responses including:

- synthesis of phytohormones acting as signaling intermediates: SA, ET, and JA;
- synthesis of phytoalexins that are low molecular weight, lipophillic, antimicrobial compounds that restrict pathogen growth;
- activation of transcription factors involved in various defense pathways that induce the expression of downstream defense genes that encode pathogenesis-related (PR) proteins (chitinases or glucanases) that possess either antifungal or antibacterial activity;
- programmed cell and tissue death (PCD) at the site of infection also called hypersensitive response (HR) which shares morphological and mechanistic features with animal apoptosis and constrains further spread of the infection by eliminating the infected host cells (Lam et al., 2001).

The system of interacting signaling pathways (Figure 2) may underscore the ability of the plant to specifically, efficiently and effectively cope with the multitude of biotic threats from its environment. It is evident the immense complexity of this signaling network, nevertheless the end result of the signal transduction is the induced production of defense proteins that directly or indirectly inhibit proliferation of the attacker. Signal-transduction pathways mediated by the plant hormones SA, JA and ET are involved in regulating appropriate defense responses. SA plays a crucial role in plant defense and is generally involved in the activation of defense responses against biotrophic and hemi-biotrophic pathogens. Increased levels of SA (endogenous induced by pathogens and exogenous applications) result in the induction of PR genes and the establishment of a state of enhanced defensive capacity, called systemic acquired resistance (SAR), that confers broad-spectrum resistance to subsequent pathogen infection (Bari et al., 2009). The SAR is a non-specific resistance that provides long-lasting protection throughout the plant, both locally and distant tissues from the site of original infection (Bent, 1996). One of the effects triggered by SA is the elicitation of an imbalance in the redox state of the cell, which results in reduction of specific disulfide bridges in the *ankyrin-repeat protein Non-expressor of PR genes* 1 (NPR1). NPR1 plays a central role in defense responses and is required for the establishment of SAR and the expression of SA-dependent defense genes like *PR-1*. Several studies indicate that JA and ET signalings often operate synergistically to activate the expression of some defense related genes after pathogen inoculation (Bari et al., 2009). JA and ET are usually associated with defense against necrotrophic pathogens and herbivorous insects. Although, SA and JA/ET defense pathways are mutually antagonistic, evidences of synergistic interactions have also been reported (Beckers and Spoel, 2006; Mur et al., 2006). This suggests that the defense signaling network activated and utilized by the plant is dependent on the nature of the pathogen and its mode of pathogenicity. Recently, JA signaling has been implicated in the long-distance information transmission (volatile signal) leading to systemic immunity in *Arabidopsis* (Truman et al. 2007). Many transcription factors involved in JA and ET signal transduction are members of the AP2/ERF group; while for example, SA signal transduction involves mostly WRKY and bZIP members. Several recent studies provide evidence for the involvement of other hormones such as abscisic acid (ABA), auxin, gibberellic acid (GA), cytokinin (CK), brassinosteroids (BR) in plant defense signaling pathways. Treatment of plants with some hormones results in the reprogramming of the host metabolism, gene expression and modulation of plant defense responses against microbial challenge. Depending on the type of plant–pathogen interaction, different hormones play positive or negative roles against various biotrophic and necrotrophic pathogens (Figure 3 in Bari and Jones, 2009). However, the underlying molecular mechanisms are not well understood and several questions remain to be answered.

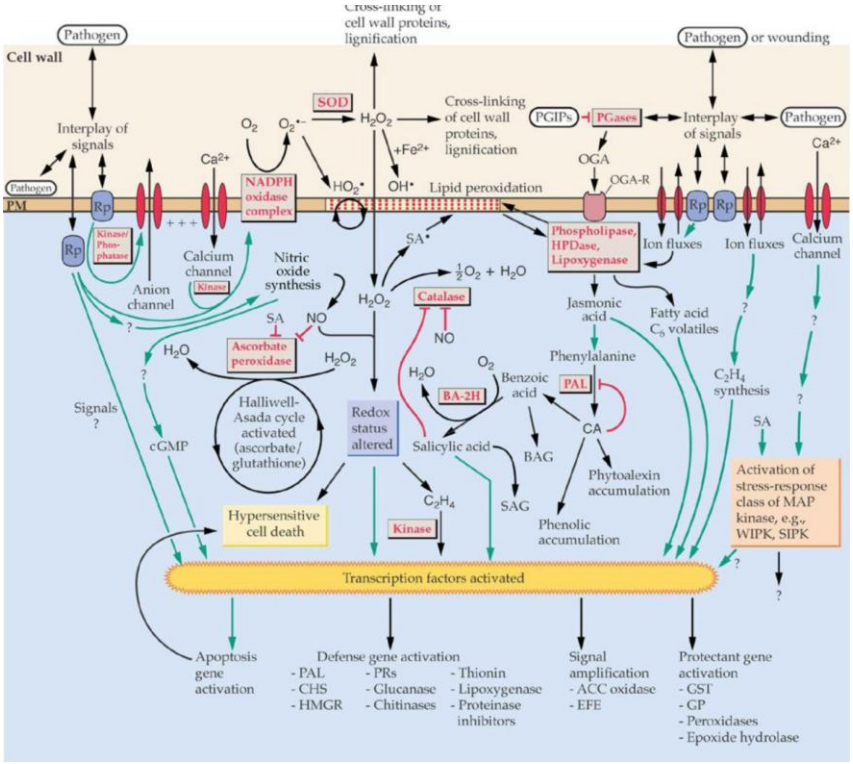
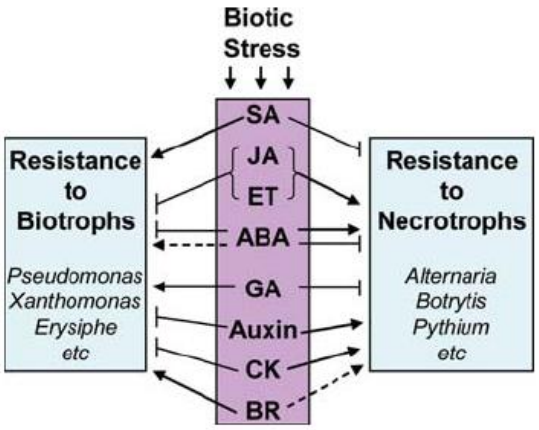


Figure 2. Complexity of signaling events controlling the defense response (Buchanan et al., 2003, in Biochemistry & Molecular Biology of Plants).



(Bari and Jones, 2009)

Figure 3. A simplified model showing the involvement of different hormones in the positive or negative regulation of plant resistance to various biotrophic and necrotrophic pathogens. The arrows indicate activation or positive interaction and blocked lines indicate repression or negative interaction.

2.3 Melon (*Cucumis melo* L.)

Melon (Cucumis melo L.), a eudicot diploid species (2n = 24 chromosomes), is one of the most important horticultural crops worldwide. It belongs to the *Cucurbitaceae* family that includes several other vegetables of economic importance such as cucumber (Cucumis sativus L.), watermelon (Citrullus lanatus (Thunb.) Matsum & Nakai), squash (Cucurbita spp.), pumpkin (Cucurbita maxima) and bottle gourd (Lagenaria siceraria). Melon is a member of the the subfamily *Melothrieae* and together with cucumbers is the major commercial vegetable crop of the genus *Cucumis*. Melon ranks as the 9th most cultivated horticultural crop in terms of total world production with 26 million of tons produced worldwide in 2009 (http://faostat.fao.org). It is mainly cultivated for the consumption of the fruits. Immature harvested fruits, not sweet are eaten raw, cooked or pickled while mature fruits with high sugar content are mainly eaten raw and marginal in cubes canned in syrup, in "fruits confits", candies, ice-creams, biscuits and also in cosmetics. Locally, seeds or leaves can also be consumed. The origin of diversity for melon was traditionally believed to be in Africa (Robinson and Decker-Walters, 1997), although recent molecular systematic studies, suggested that it might be originated from Asia and then reached to Africa (Renner et al., 2007). Melon made its first appearance in Italy during the period of the Roman Empire (Pangalo, 1929) and can be considered as the most highly developed types of ancient cultivated species (Mallick and Masui, 1986; Manniche, 1989). The high polymorphism of cultivated melons has led to propose different classifications recently focused mainly on central Asian diversity (Pitrat et al., 2008). It comprises eight cultivated subspecies, namely, vars. *cantalupensis, chito, conomon, dudaim, inodorus, flexuosus, reticulatus* and *makuwa* (Choi et al., 2012) and of these, the cantaloupe melon (Cucumis melo L. var. cantalupensis), with a rough and warty skin, not netted, is considered of great commercial interest. Melon, due to its high economic value worldwide, short generation time, relatively small genome, and highly phenotypic polymorphism, especially in vegetative and fruit morphology, is an attractive model species for genetic and molecular studies (Liu et al., 2004; Monforte et al., 2004; Sestili et al.,

2014). The amount of genomics information has been increasing in melon, due to the broad range of genomic tools developed (Ezura and Fukino, 2009) such as genetic maps (Gonzalo et al., 2005, Deleu et al., 2009, Harel-Beja et al., 2010), a detailed physical map (González et al., 2010), the bacterial artificial chromosome (BAC) libraries (Van Leeuwen et al., 2003), an oligo-based microarray (Mascarell- Creus et al., 2009, Ophir et al., 2010), TILLING and EcoTILLING platforms (Nieto et al., 2007, Dahmani-Mardas et al., 2010, González et al., 2011), several large expressed sequence tag (EST) datasets (Gonzalez-Ibeas et al., 2007, Clepet et al., 2011) and development of mapping populations (Perin et al., 2002, Eduardo et al., 2005, Fernandez-Silva et al., 2008, Paris et al., 2008). The full sequence of melon genome is readily available and greatly facilitates the identification of genes underlying certain traits and the elucidation of mechanisms that regulate relevant characteristics (Garcia-Mas et al., 2012). Furthermore, high-throughput transcriptome approaches using novel sequencing technologies have been used in melon to generate Single nucleotide polymorphism (SNPs) and simple sequence repeat (SSR) markers useful for molecular breeding (Blanca et al., 2011, Esteras et al., 2013) and for discovering genes associated with major fruit metabolic pathways, fruit ripening and abiotic stress (Dai et al., 2011, Portony et al., 2011, Gonzalez-Ibeas et al., 2011, Corbacho et al 2013, Chen et al., 2014). The increasing availability of genome sequences from higher plants provides us with an important tool for understanding plant evolution and the genetic variability existing within cultivated species. The use of the genome sequence in future investigations will facilitate the understanding of Cucurbits evolution and the improvement of breeding strategies.

2.4 *Fusarium oxysporum* f. sp. *melonis* (FOM)

Fusarium oxysporum Schltdl. is a fungal soil borne facultative parasite worldwide. The species includes non-pathogenic and pathogenic strains, the latter causing vascular wilt and root rot on many economically important crops (Haegi et al., 2013). Despite *F. oxysporum* causing destructive vascular wilts in a wide variety of crops (approximately 80 botanical species), strains have been subdivided into over 100 different host-specific forms (formae speciales) which are morphologically indistinguishable and represent intraspecific groups of strains with similar or identical host ranges (Haegi et al., 2013). A *forma specialis* can be further subdivided into races based on characteristic virulence patterns on differential host cultivars (Gordon and Martyn, 1997). The infection process of *F. oxysporum* involves the following steps: spores germinate in response to root exudates, produce penetration hyphae that attach to the root surface and penetrate it directly, and grow invasively in the water-conducting vessels of the host following by yellowing of leaves and successively death of the entire plant (Rodriguez-Gàlvez and Mendgen, 1995). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melonis* W. C. Snyder & H. N. Hansen (FOM) is the most destructive and less controllable damaging disease of melon worldwide (Figure 3). It occurs throughout Italy (Belisario et al., 2000) and Europe (Gomez Vazquez and Tello Marquina, 2000) as well as North and Central America (Zuniga et al., 1997), Asia (Namiki et al., 1998) and Africa (Schreuder et al., 2000). Plants may be attacked at any stage of growth although they are mainly susceptible to the fungus at the early stage, from the cotiledonary leaves to the first true leaf (Belisario et al., 2000; Ficcadenti et al., 2002). Once introduced into the field, FOM can persist in soil over extended periods on crop residues and non-host crops, by forming chlamydospores (Haegi et al., 2013). Chemicals, such as methyl bromide, acetochlor treatment and dinitroaniline herbicides, can reduce disease incidence but often cause an unbalance in the soil microbiological flora and have a negative effect on hygienic-sanitary conditions of consumers. The use of dry mycelium of *Penicillium chrysogenum* is a

good way to induce resistance against root disease such as FOM (Dong and Cohen, 2001). Grafting represents an effective tool for controlling the disease, but its cost and time consumption limit the use to greenhouse production only (Marukawa, 1979; Perchepied and Pitrat, 2004). Therefore, the most effective and sustainable strategy to prevent this disease is through genetic resistance. Four races of the pathogen (races 0, 1, 2, and 1.2) have been defined according to host resistance genes overcome by variants of the pathogen (Risser et al., 1976). Two dominant independently inherited resistance genes, *Fom-1* and *Fom-2*, provide long-lasting stable resistance to races 0 and 2 and races 0 and 1, respectively (Risser et al., 1976). Race 0 incites disease on melon genotypes that lack genes for resistance to FOM. *Fom-1* and *Fom-2* are independently inherited dominant genes in muskmelon that control resistance to races 0 and 2, and races 0 and 1, respectively (Risser et al., 1976; Zink, 1992). The presence of these genes in a plant confers a high level of resistance to races 0, 1 and 2 (Risser et al., 1976). *Fom-3* and the recessive *fom-4* genes have been reported to confer resistance to races 0 and 2 in two different melon cultivars (Oumouloud et al., 2010). *Fusarium oxysporum* f. sp. *melonis* race 1.2 (FOM1.2), overcoming the resistance genes, represents the most widespread and harmful race causing economic losses up to 100% of melon yield (Luongo et al., 2014). The resistance towards FOM1.2 is a horizontal resistance and is probably based on the appearance of the SAR that is correlated with genes encoding proteins involved in defense mechanism conferring high level of resistance towards different pathogens or secondary infections. Resistance to race 1.2 is complex, being controlled by multiple recessive genes and strongly affected by environment (Chikh-Rouhou et al., 2011; Zvirin et al., 2010). Two complementary recessive genes, conferring a near-complete resistance to race 1.2, were identified in the breeding line "BIZ" (Herman and Perl-Treves, 2007). Furthermore, nine loci and a major recessive quantitative trait loci (QTL) linked to FOM1.2 resistance were also reported (Herman et al., 2008; Perchepied et al., 2005). At present, only partially resistant genotypes and some *inodorus* accessions of Western origin are commercially available mainly as rootstock (Luongo et al., 2014; Oumouloud et al., 2009). Partial resistance to FOM1.2, found in several Far-Eastern lines (Ogon 9 and Kogane-Nashi Makuwa), was introgressed into the cultivar "Isabelle" from which the doubled haploid (DH) resistant line NAD, used in this work, was derived by means of parthenogenesis induced *in situ* by pollen denatured with high doses of gamma rays. This led to the destruction of the generative function of pollen, but do not affect its capability in egg-cell stimulation, allowing the formation of parthenogenic embryos (Ficcadenti et al., 1995a). Oumouloud et al. (2013) hypothesized that "BIZ" and "Isabelle" might carry different loci for resistance and not just different alleles in similar loci. The DH line NAD showed a more resistance level to FOM1.2 than other genotypes and the mother "Isabelle", since the homozygous status of the resistance genes in these lines allows full expression of the polygenic recessive resistance to the fungus (Ficcadenti et al., 2002). It therefore represents a powerful material for genetic improvement, either to hasten the selection of resistant cultivars or to study the molecular basis of the resistance towards this dreadful pathogen.

Figure 3. Symptoms of Fusarium wilt in melon

2.5 RNA Sequencing

The advent of next generation sequencing (NGS) technologies has marked a new era of transcriptomics (Egan et al., 2012; Hamilton and Buell, 2012). NGS is underpinning an ongoing revolution in the life sciences and it is now difficult to identify areas of biology that are not already being profoundly affected by the massive amounts of high quality DNA sequence information that has been generated cost-effectively and efficiently. Plant biology is naturally no exception to this revolution; indeed the ease of genetic analyses in many plant species and the value of crop species have made plant science an especially fertile area for many of the "omics" technologies (Martin et al., 2013). The transcriptome sequencing of an organism provides quick insights into the gene space, opportunity to isolate genes of interest, development of functional markers, quantification of gene expression, and comparative genomic studies (Garg and Jain, 2013). RNA Sequencing (RNA-Seq) is increasingly used for gene expression profiling in plants as it provides significant advantages over traditional microarray analysis, e.g. accurate quantification of gene expression with low background, high sensitivity and the capability of detecting differential expression over a large dynamic range, high reproducibility for both technical and biological replicates as well as the possibility of detecting novel splicing isoforms and boundaries of un-translated regions at single nucleotide resolution (Bagnaresi et al., 2012). RNA-Seq approaches have an open architecture, meaning that they are not restricted to detecting only those transcripts that are represented on microarrays, and exhibit more extreme upper and lower limits of detection, which allow more accurate quantification of differential transcript expression, as well as the identification of low-abundance transcripts. Furthermore, no previous genome sequence knowledge is necessary, as RNA-Seq data sets themselves can be used to create sequence assemblies for subsequent mapping of RNA-Seq reads. An important issue that still needs to be addressed is the inherent bias introduced by the different steps of library construction and so the tantalizing prospect of direct RNA-Seq (Ozsolak and Milos, 2011) has great promise in this regard. Although becoming cheaper, transcriptome sequencing remains an expensive endeavour. Furthermore, the assembly of millions and billions of RNA-Seq reads to construct the complete transcriptome poses great informatics challenges. RNA-Seq analysis has been used to study several plant-fungal interaction (Bagnaresi et al., 2012; De Cremer et al., 2013; Gao et al., 2013; Gusberti et al., 2013; Meinhardt et al., 2014) and in particular, in the

context of *Fusarium oxysporum* infection, it has been performed in *Arabidopsis* (Zhu et al., 2013) and banana (Guo et al., 2014; Li et al., 2012).

2.6 *In vitro* culture of *Cucumis melo* L.

Biotechnology tools as genetic engineering, molecular biology and tissue culture techniques are able of surpassing the natural genetic barriers leading to improved plant material and allow characterizing important horticultural traits. The establishment of an efficient and stable regeneration methodology to recover viable plants is an essential requirement for successful application of genetic engineering techniques that represent an essential step for functional genomics studies. In melon, although several *Agrobacterium tumefaciens*-mediated procedure have been reported to validate the functions of isolated melon genes (Nunez-Palenius et al. 2006; Chovelon et al. 2011; Choi et al. 2012; Finco et al. 2013), the establishment of efficient regeneration and genetic transformation techniques is needed, due to the huge amount of newly developed genomic sequence information. Although there are several reports regarding a wide range of melon cultivars, *in vitro* regeneration by organogenesis or somatic embryogenesis is not easy in melon due to several problems, e.g. the great number of tetraploid plants regenerated, (Ficcadenti and Rotino, 1995; Guis et al. 2000; Akasaka-Kennedy et al. 2004; Yalcin-Mendi et al. 2004; Castelblanque et al., 2008; Sebastiani et al., 2013). Successful regeneration systems depend on many factors, such as genotype, explants source, gelling agent and hormone concentration. Shoot formation from cotyledons, hypocotyls, roots, or leaf explants has been obtained in melon with a different procedures and frequency rate (Moreno et al., 1985; Dirks and Buggenum, 1989; Curuk et al., 2002; Ntui et al., 2009; Thiruvengadam et al., 2010; Choi et al., 2012). Melon regeneration is strongly genotype dependent and sexually transmissible (Ficcadenti and Rotino, 1995) and the efficiency is relatively low. However, environmental and hormonal requirements for melon regeneration continue to be poorly understood and developing simple and routine procedures to regenerate and transform all melon genotypes is still challenging. Beneficial effects of benzylaminopurine (BAP) or kinetin in combination with indoleacetic acid (IAA) on shoot induction have been observed in melon by several authors (Moreno et al. 1985, Kathal et al. 1986; Niedz et al. 1989; Choi et al. 2012; Sebastiani et al., 2013). To increase the plant regeneration rate, reduce the frequency of escapes and limit the influence of biological and physical factors, that make the established protocols not necessarily applicable to other melon cultivars (Guis et al., 2000; Galperin et al., 2003; Pech et al., 2007; Nunez-Palenius et al., 2008), an alternative regeneration system is needed. The study of the regeneration potential of a wider range of melon cultivars could help to increase the efficiency of cell transformation and plant regeneration from transformed cells. A great problem in tissue culture technique is the so called "somaclonal variation" (Larkins and Scowcroft 1981) for which a consistent proportion of the regenerated plants genetically differ from the original parental type. The presence of somaclonal variation has been reported for many plant species and has potential application to establish a large number of new breeding lines (Ezura 1994, 1995; Rani et al. 2000; Sultana and Mahabubur, 2014). The presence of somaclonal variation among the clones of a single parental line is a negative effect since it is often heritable, thus the maintenance of the genetic integrity of explants with respect to the mother plants is crucial during *in vitro* culture (Ngezahayo et al., 2007; Jin et al., 2008). The molecular markers were extensively used in germplasm characterization, fingerprinting, genetic analysis, linkage mapping and molecular breeding. Among molecular markers, random amplified polymorphic DNA (RAPDs) are widely used in studying the genetic diversity of somaclonal variants in several plant species such as banana

(Sheidai et al., 2008), *Centaurea ultreiae* (Mallon et al., 2010), *Eucalyptus tereticornis* (Aggarwal et al., 2010), *Withania coagulans* (Jain et al., 2011) and *Desmodium gangeticum* (Cheruvathur et al., 2013). RAPD analysis allows the amplification of discrete fragment of the genome without the previous knowledge of its sequence and is technically simple, quick to perform, requires very little plant material and yields true genetic markers (Adhikari et al., 2004). The production of stable transgenic melon plants expressing target genes is a bottleneck for melon functional genomics study, thus the development of a suitable plant regeneration system will hasten either the breeding or the functional studies on this specie also in relation to the accumulation of genomic sequence information and the identification of unique melon genes with unknown functions.

3. AIM OF THE STUDY

The aim of this Ph.D. work was to study the melon-FOM1.2 pathosystem carrying out a RNA-Seq analysis to investigate the dynamic changes of the melon defense transcriptome in infected and healthy plants in response to FOM1.2 inoculation and to gain new insights on genes underlying the resistance mechanism against this dreadful pathogen. Furthermore, for future functional studies of the candidate resistance genes obtained from the transcriptome analysis, melon *in vitro* regeneration and transformation systems were developed.

4. RESULTS AND DISCUSSION

TRANSCRIPTOME ANALYSIS

4.1 Phenotypic screening of the disease

Fusarium wilt progression in the resistant DH line NAD and in the susceptible cultivar Charentais-T (CHT) plants inoculated with FOM1.2 was monitored by phenotypic screening at 8, 15, 21 and 35 days post inoculation (dpi). The response to infection is affected by the inoculum concentration and time of exposure, the virulence of strain, the age of the plant and the type of substrate for plant growth (Sestili et al., 2011). Symptoms as necrosis and severe lesions on plant collars became obvious in the compatible interaction already before 8 dpi; supporting the validity of the inoculation procedure (Figure 4b). The 70% and the 90% of CHT plants dead at 15 and 21 dpi respectively, due to the massive increase of fungal biomass in the xylem sap. Despite colonization, NAD plants remained healthy, free of wilt symptoms during all the time course experiment (35 dpi), confirming its high level of resistance (Ficcadenti et al., 2002) (Figure 4a).

Figure 4. Interaction between melon and FOM1.2: a) incompatible (NAD/FOM1.2); (b) compatible (CHT/FOM1.2).

4.2 Melon colonization by FOM1.2

In this study a FOM1.2 - green fluorescent protein (GFP) expressing strain was used to compare fungal colonization in susceptible CHT *vs* resistant NAD melon genotypes. Similar to other fungal pathogens, the early stages of interaction between *F. oxysporum* and the host are crucial for the outcome of infection (Ruiz-Roldan et al., 2010). Key processes occurring during these initial stages include spore germination, adhesion to the host surface, establishment of hyphal networks through vegetative hyphal fusion, differentiation of infection hyphae, and penetration of the host (Tucker et al., 2001). To explore infection patterns in barley and in the *Arabidopsis* model system, the green fluorescent protein gene (*gfp*) was used to transform *F. graminearum* (Skadsen et al., 2004). The infection of xylem-colonizing FOM strains and FOM1.2 in particular has been documented in melon by using the GFP (Di Pietro et al., 2003; Inoue et al., 2002; Zvirin et al., 2010). The expression of GFP does not affect the morphology of the fungus, growth rate, pigmentation, colony morphology and conidiation, and the transgenic strains are as infective as the wild type isolate (Zvirin et al., 2010). Fluorescence intensity, being correlated with the amount of protein produced, is used for easy quantification of fungal biomass and estimation of disease levels (Maor et al., 1998). To observe the invasive growth and colonization of NAD and CHT plants, sections from roots and crowns of plants inoculated with the GFP-expressing fungus were prepared at 24 and 48 hours post inoculation (hpi). During the course of experiment, all seedlings appeared healthy, without any visible disease symptoms. Fluorescence microscopy observation readily detected that during early stages of infection FOM1.2 adheres to the root surface, differentiates infection hyphae and directly penetrates the root (Figures 5-6-7). Figures 5a and 5b show that at 24 hpi FOM1.2 was massively observed only on the root surface of the two plants where the hyphae start to grow. A large quantity of fungal biomass was detected over the root of CHT (Figure 5b), but the way and the timing of melon colonization (germination rate and the detection of hyphae in melon tissues) were comparable in both genotypes. Mycelial structures were observed within the plant tissue at 48 hpi (Figures 5c, d). Figure 6a shows FOM1.2 entering the root epidermis. FOM1.2 was capable of invading the epidermal cells of melon roots directly and fungal hyphae were able to penetrate cell walls to grow inside (Figures 6b, c) and outside cells (Figure 7a). At 48 hpi, the fungus grew on the root epidermis (Figure 7a) and adhered to epidermal cell borders. The mycelium crossed the cortex and endodermis through narrow pores in cell walls and reached xylem vessels, where it sporulated and produced secondary hyphae that grew upwards (Figures 7b). Only isolated cells were recovered in crown sections at 48 hpi (Figure 7c). These data are in contrast to what previously reported by Sestili et al. (2011) that indicated a more rapid and continuous colonization of the stem already at 1 and 2 dpi. FOM1.2 colonized the resistant plant's vascular system, but the incidence of seedling infection was lower than in susceptible CHT genotype, suggesting stronger defense responses in NAD that dampen fungal virulence at the pre-xylem stage of infection. This was observed also in Zvirin et al. (2010) for the resistant melon line "BIZ" that was colonized by the pathogen, but as NAD was able to quantitatively inhibit fungal progression by expressing an efficient defense response. This result indicated that the resistant plant have a basal defense machinery underlying host defense that lead to contrast FOM1.2 virulence, but also to tolerate the fungal growth. Further and deeper investigations on pathogen invasion during the whole life cycle of the resistant plant (from seedling to mature plant) in a natural infection regime will be useful to discover either the way used for tolerating FOM1.2 infection or the plant section where the fungal progression is reduced.

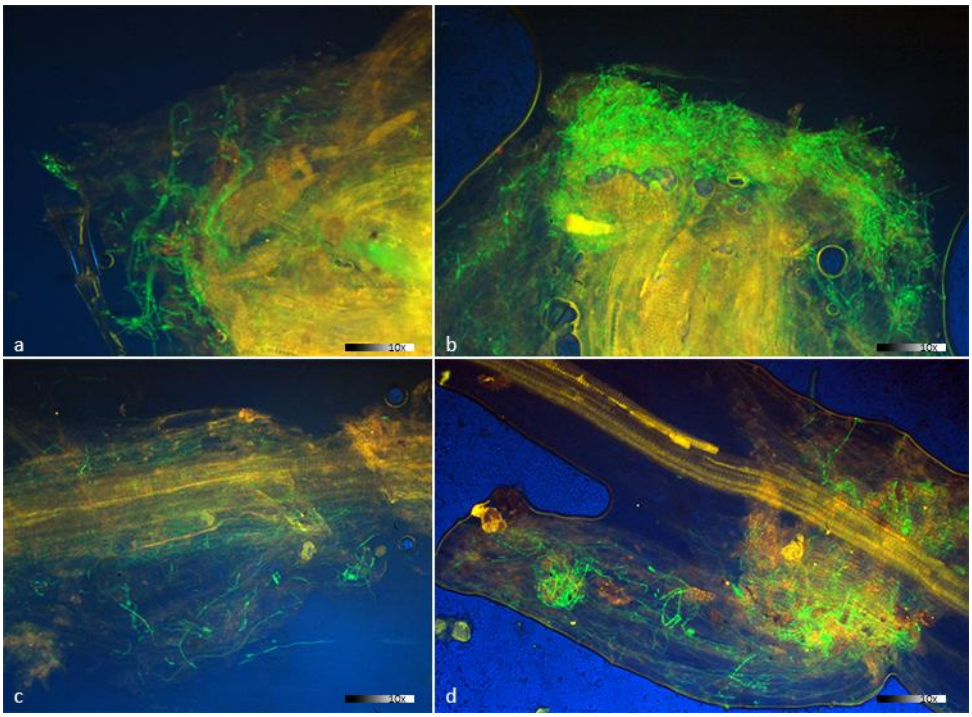


Figure 5. Fluorescence photographs of early stages of infection (24 and 48 hpi) of resistant NAD and susceptible CHT melon genotypes by FOM1.2 expressing a *gfp* reporter gene. a, b) At 24 hpi mycelium densely covering the main root of NAD and CHT, respectively. c, d) At 48 hpi FOM1.2-GFP hyphae growing over the epidermis in NAD and CHT, respectively.

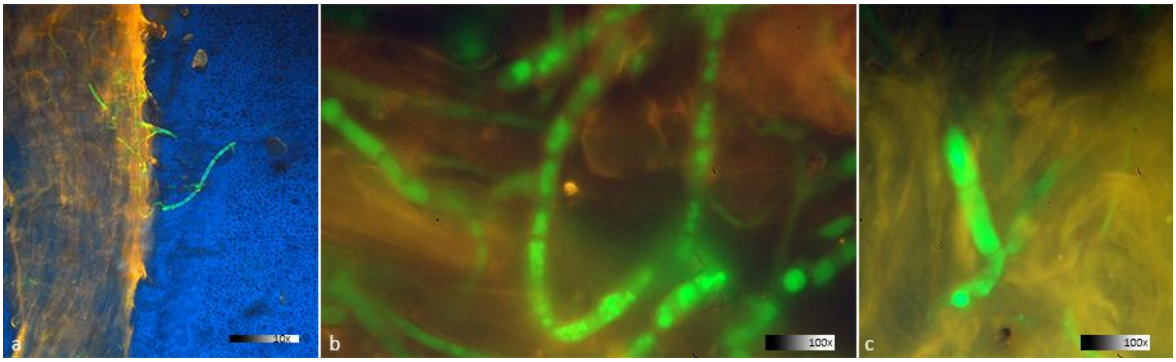


Figure 6. a) Hypha penetrating an epidermal root cell at 48 hpi. b, c) Germinating microconidia in the root cells.

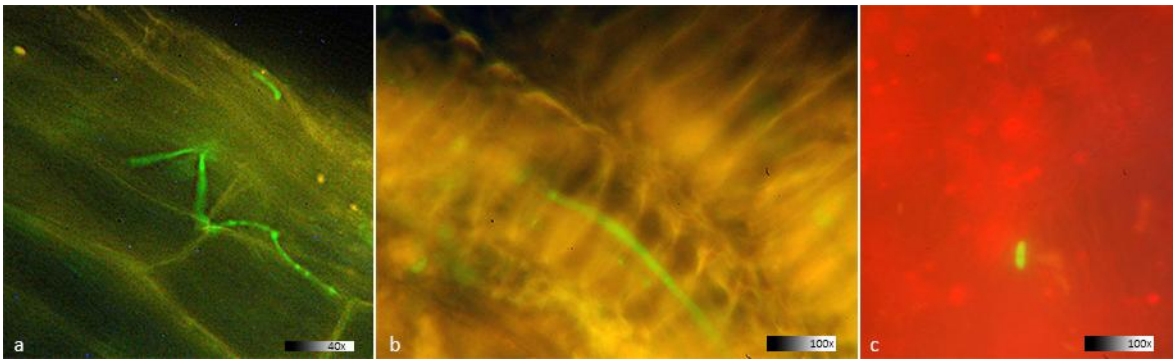


Figure 7. a) Hyphae attached and growing along the root epidermal cells. b) Hypha growing confined in a xylem vessel. c) A single germinating microconidium recovered in crown sections at 48 hpi.

4.3 Read number, transcriptome coverage and total expressed genes

RNA-Seq analysis was carried out on cDNA samples from both resistant NAD and susceptible CHT melon genotypes infected with FOM1.2 in order to identify differentially expressed genes (DEGs) putatively involved with the infection process and resistance response. Stem samples of infected plants and the corresponding water-treated controls (mock infection) were collected at 24 and 48 hpi, since the transcriptional changes associated with resistance responses occur within the first 2 dpi and are maintained with few changes thereafter (Sestili et al., 2011). Most transcriptomics studies involving *Fusarium oxysporum* have focused on the interactions that occur in the xylem, and these studies suggest that the main resistance responses occur within or along the vessels (Sestili et al., 2011). Illumina GAIIx reads ranging from 12 to 25 million were obtained from 16 samples (51 bases, single-end; on average 18.5 million reads) (Table 1). Two biological replicates for each genotype (NAD and CHT, 24 and 48 hpi sampling time points and mock-inoculated vs FOM1.2-infected treatment) were used according to recommended RNA-Seq standards (Encode project, 2011). The fastQC application was employed to detect sequence contaminants. Low quality reads (quality <= 10 phred score) and contaminants were trimmed out with Cutadapt software (Martin, 2011). Contaminant-free, filtered reads were mapped with Bowtie/TopHat to the *Cucumis melo* genome (Garcia-Mas et al., 2012). Raw read counts were obtained from BAM alignment files by counting with HTSeq software (Anders et al, 2014). An RPKM (Reads per Kilobase per Million) cutoff value of 0.5 was set to declare a locus expressed, resulting in 19162 and 18615 loci above the expression cutoff for NAD and CHT, respectively. Pearson correlations between replicates were always above 0.9 and samples undergoing the same treatment clustered together (Figure 8).

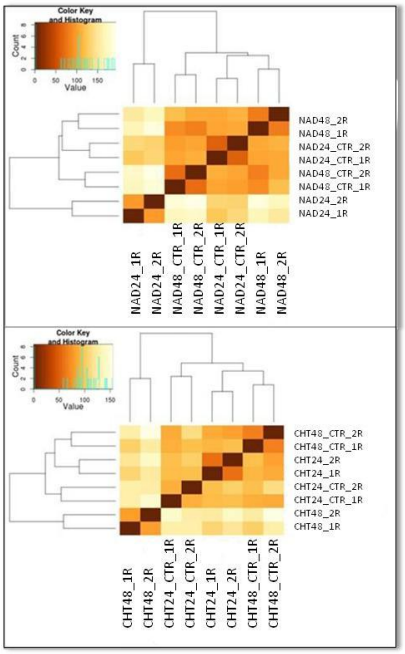


Figure 8. Pearson correlations and sample clustering of the two genotypes.

Table 1. Read and alignment data

Sample	Illumina passed-filter	Contaminant free	Total alignments	Mapped reads	Uniquely mapped reads
NAD_ctr_24h_1R	19,181,676	18,871,022	19,155,684	18,223,397	17,589,670
NAD_ctr_24h_2R	20,514,891	20,284,245	20,255,130	19,570,795	19,108,335
NAD_inf_24h_1R	22,110,709	21,750,829	21,815,220	20,602,921	19,876,896
NAD_inf_24h_2R	19,877,440	19,436,342	19,374,115	18,531,325	17,962,969
NAD_ctr_48h_1R	18,764,296	18,496,988	18,266,326	17,818,229	17,511,180
NAD_ctr_48h_2R	15,766,584	15,400,859	15,301,821	14,829,214	14,505,549
NAD_inf_48h_2R	20,355,344	20,020,385	19,661,240	19,166,623	18,815,392
NAD_inf_48h_2R	25,353,337	24,743,002	24,466,362	23,626,737	23,010,080
CHT_ctr_24h_1R	16,723,007	16,393,253	17,186,276	15,366,305	14,254,086
CHT_ctr_24h_2R	16,483,206	16,280,414	16,263,182	15,620,871	15194697
CHT_inf_24h_1R	14,620,721	13,970,663	13,684,940	13,317,799	13,061,691
CHT_inf_24h_2R	16,750,447	16,541,183	16,330,573	15,915,948	15,625,369
CHT_ctr_48h_1R	16,741,386	16,586,085	16,393,568	16,010,067	15,746,764
CHT_ctr_48h_2R	22,366,924	22,134,163	21,937,793	21,390,345	21,025,848
CHT_inf_48h_1R	12,888,107	12,271,022	12,457,799	11,345,070	10,639,496
CHT_inf_48h_2R	16,750,542	16,447,943	16,105,086	15,659,318	15,358,087

4.4 Differentially expressed genes and overrepresented categories

The R package DESeq (Anders and Huber, 2010) was employed for DEGs calling. The False Discovery Rate (FDR) threshold was set to 0.05. A total of 5873 DEGs was functionally annotated. The resistant DH line NAD, inoculated with FOM1.2, showed 2461 and 821 DEGs, of which 2023 (82.2%) and 568 (69.2%) were up-regulated at 24 and 48 hpi, respectively (Table 2). This approximately 3-fold ratio was reversed in the susceptible genotype CHT, in which 882 and 2237 DEGs were identified at 24 and 48 hpi, respectively; among these, only 253 (28.7%) were up-regulated at 24 hpi, whereas 1810 (80.9%) were up-regulated at 48 hpi (Table 2). Upon pathogen infection, there is activation of cellular responses in attacked cells of both susceptible and resistant plants. However, in NAD cellular defense responses are induced more rapidly and stronger than in CHT, confirming that the timely recognition of the invading FOM1.2 combined with the rapid and effective induction of defense responses makes a key difference between resistance and susceptibility. The establishment of compatibility is characterized by a progressive increase of the number of genes involved, underlying the significant metabolic disturbances that might be associated with symptoms development. An analogous enrichment in down-regulated genes was observed during the compatible plant-pathogen interaction of cotton roots with *Fusarium oxysporum* f. sp. *vasinfectum*, particularly at early stages of infection (Dowd et al., 2004). The DEGs distribution and their reciprocal ratio at the two time points are highlighted in the MA-plots (Figure 9). The VENN diagram showed that only 79 DEGs resulted to be in common between the two genotypes, when pooling the whole set of DEGs for each variety and both time points (Figure 10).

Table 2. Accounting for DEGs in the two types of interactions

DESeq2*	NAD_24h CTR/STRESS	NAD_48h CTR/STRESS	CHT_24h CTR/STRESS	CHT_48h CTR/STRESS
TOT DEGs	**2461**	**821**	**882**	**2237**
UP-REGULATED GENES	2023	568	253	1810
DOWN-REGULATED GENES	438	253	629	427
% UP-REGULATED GENES	82.2	69.2	28.7	80.9
% DOWN-REGULATED GENES	17.8	30.8	71.3	19.1

**The False Discovery Rate (FDR) threshold was set to 0.05*

CTR: control plants; STRESS: stressed i. e. incoculated with FOM1.2

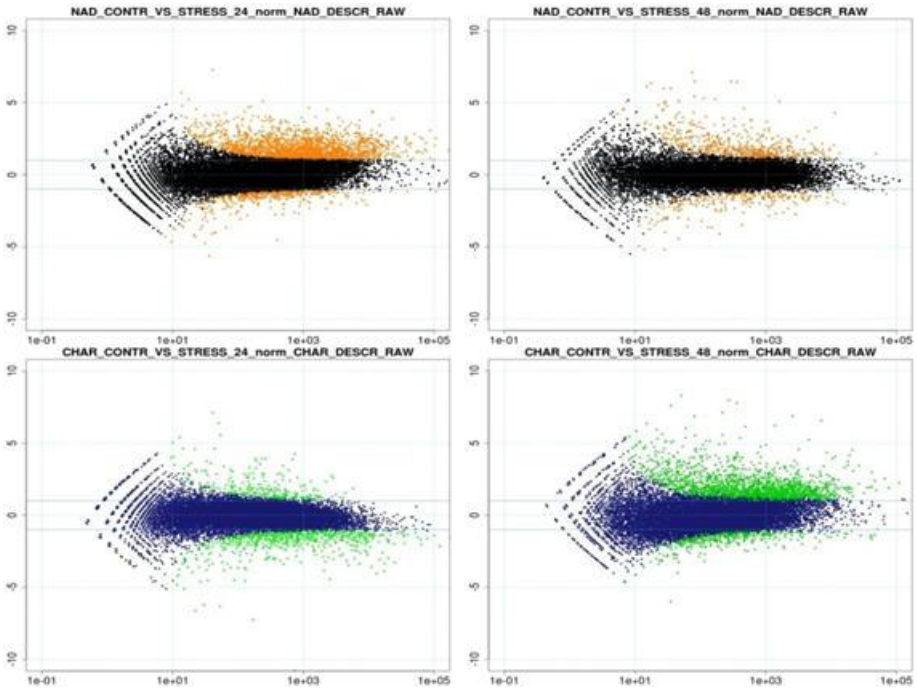


Figure 9. MA-plots accounting for the distribution of DEGs over the two genotypes.

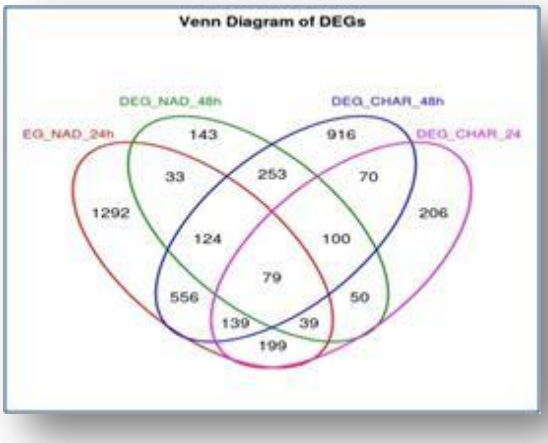


Figure 10. Venn diagram showing the relationships between the total DEGs in the two genotypes after FOM1.2 inoculation.

4.5 Analysis of GO enriched categories

To gain insights into the functionality of the genes responsive to FOM1.2 infection, gene ontology (GO) enrichment analysis for the genes up- or down-regulated in both genotypes and at each time point was performed. GO enrichment analysis in RNA-Seq experiments poses specific challenges as the status of modulated gene (i.e. DEG classification) is related to the read counts and thus biases in favour of longer and highly expressed genes are expected (Young et al., 2010). The goseq bioconductor package (Robinson and Oshlack, 2010) was used to account for RNA length bias typical of RNA-Seq approaches (Oshlack and Wakefield, 2009). A total of 5155 GO annotations were retrieved. The DEGs called by DESeq package were used as a test set for goseq input. Goseq output (threshold FDR=0.05) yielded 27 GO terms enriched in both genotypes, although activated at different times, that were consistent with response to fungal infection, including "defense response to fungus" (GO:0050832), "response to chitin" (GO:0010200), "plant-type cell wall" (GO:0009505), "respiratory burst involved in defense response" (GO:0002679), "regulation of plant-type hypersensitive response" (GO:0010363), "systemic acquired resistance, salicylic acid mediated signaling pathway" (GO:0009862). The GO groups in common between the two genotypes included different number of DEGs and a contrasting pattern of transcript accumulation. In the group "defense response to fungus" (GO:0050832) enriched at 48 hpi, 25 and 45 genes were up-regulated in NAD and CHT, respectively. The *class i chitinase gene* (MELO3C007962) was shared between the two genotypes, with a higher log_2-fold change (log_2-fc) in the resistant plant (log_2-fc=4.62), while the *flavin-containing monooxygenase 1-like gene* (MELO3C015551) was expressed only in NAD at 48 hpi (Table 4). Over-representations of GO terms in the set of differentially regulated genes were evaluated to indicate which biological processes, molecular functions and cellular components were most affected after FOM1.2 infection. However, despite these similarities, careful consideration of the individual genes contributing to the common enriched GO terms revealed substantial diversity between genotypes. Several GO terms resulted to be genotype- and time point-specific (Figure 11). In CHT, the GO terms accounted for the biological process, molecular function and cellular component categories were always more depicted at 48 hpi than at 24 hpi (44 vs 19). These results indicated a different response of CHT at the two time points due to the increasing fungal colonization during the infection. At early time point, the susceptible plant undergoes mainly nutritional/metabolic changes, e.g. "nitrate assimilation" (GO:0042128), "response to sucrose stimulus" (GO:0009744), "tyrosine metabolic process" (GO:0006570) and "photosynthesis" (GO:0015979), while at 48 hpi the plant, deeply colonized by the fungus, switch on senescence pathways and triggers detoxification and protection processes for going through the disease, e.g. "ethylene mediated signaling pathway" (GO:0009873), "proteasome core complex assembly" (GO:0080129) and "ubiquitin-dependent protein catabolic process" (GO:0006511), "glutathione metabolic process" (GO:0006749), "peroxisome" (GO:0005777). Examination of GO terms suggested that a large part of the NAD transcriptome is devoted to control defense mechanisms as revealed by the 33 specific-GO terms enriched exclusively at 24 hpi (Figure 12), of which "defense response, incompatible interaction" (GO:0009814), "plant-type cell wall organization" (GO:0009664) "hyperosmotic response" (GO:0006972), "auxin polar transport" (GO:0009926), "cellulase activity" (GO:0008810) emerged as major effectors of resistance to FOM1.2 (Figure 12). Of note, in the group "defense response, incompatible interaction" (GO:0009814) (8 genes), the *pectate lyase 22-like* (MELO3C02447) and the *pectate lyase 12-like* (MELO3C002319) *genes* were specifically expressed only in NAD at 24 hpi and the *E3 ubiquitin-protein ligase atl6-like gene* (MELO3C004286) were up-regulated (Table 4), supporting their role in eliciting plant defense response (Wegener et al., 2001; Craig et al., 2009). Among the 18 NAD specific GO terms at 48 hpi the elements "cellular response to stress" (GO:0033554), "regulation of hydrogen peroxide metabolic process" (GO:0010310), "oxylipin biosynthetic process"

(GO:0031408), "response to jasmonic acid stimulus" (GO:0009753) and "calmodulin binding" (GO:0005516) deserved particular attention. These GO groups, being NAD-specific, are promising candidates for revealing genes underpinning NAD resistance. The "pectinesterase activity" (GO:0030599) and "manganese (Mn) ion binding" (GO:0030145) resulted the only NAD GO terms in common between the two time points considered. A key role of antioxidative systems in plants in relation to high Mn amounts has also been reported as a defense mechanism. Mn frequently induces oxidative stress, and then several defense enzymes and antioxidants are stimulated to scavenge the superoxide and hydrogen peroxide formed under stress (Yang et al., 2007).

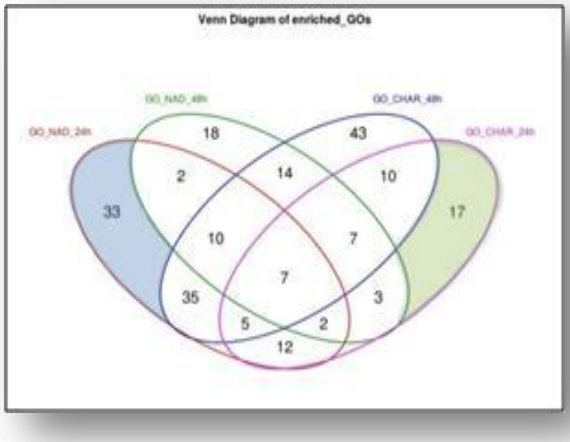


Figure 11. Venn diagram showing the relationships among the total enriched GO terms in the two genotypes after FOM1.2 inoculation.

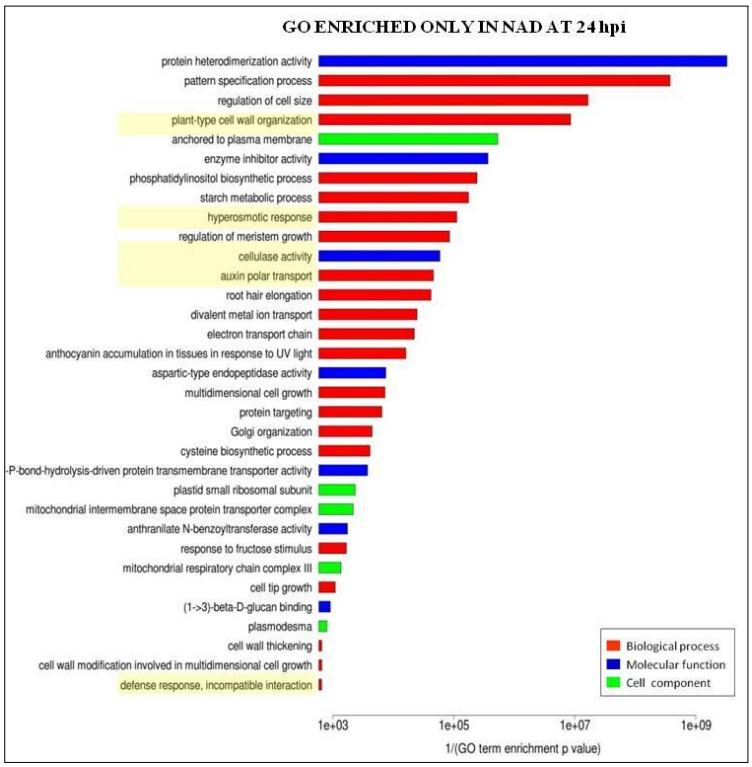


Figure 12. GO terms enriched only in the resistant genotype NAD at 24hpi. Terms highlighted in yellow represent the most relevant GO groups.

4.6 Analysis of DEGs by comparing the mock-inoculated tissues of the two genotypes

NAD and CHT genotypes both belong to the botanical variety *cantalupensis*. The plants have no detectable morphological variants and resulted genetically similar (identical) using ISSR molecular markers (Sestili et al., 2005). The essential (unique) difference between NAD and CHT is the respective resistance or susceptibility to all races of FOM. Therefore, to study the heritability of the resistance to FOM1.2 these two genotypes were chosen for developing two F2 segregant populations (Sestili et al., 2005). In particular, NAD being a DH line carries out all the genetic characters in homozygosis and ensures the expression of all the recessive loci, e.g. the resistance to FOM1.2. To assess if there are differences in the basal transcriptionally expression of the two plants the transcriptomes of the mock-inoculated samples (NAD vs CHT) were also studied. The analysis of DEGs by comparing the mock-inoculated tissues data of the two genotypes led to the identification of "response to stress" (GO:0006950) and "defense response" (GO:0006952) specific NAD enriched GO terms at both time points, and "response to chitin" (GO:0010200) and "response to biotic stimulus" (GO:0009607) groups enriched only at 48 hpi. This suggested that the healthy resistant plant NAD exhibits a basal defense machinery that is likely to contrast FOM1.2 infection. Based on this data the transcriptomes of the mock-infected plants were studied to analyze the expression of defense genes at the two time points considered. Table 3 reports selected melon genes that showed very high expression value in NAD and coding for proteins notoriously implicated in the resistance to fungal pathogens. Five *WRKY genes* were strongly up-regulated at 24 hpi in NAD vs CHT (Table 3). *WRKY genes* are also known to induce several pathogenesis related genes (Peng et al., 2010) and this may account for the enrichment of the GO term "defence response" in melon. Previous microarray analyses showed activation of several *WRKY genes* as induced by *M. oryzae* and/or chitin oligosaccharide elicitors (e.g. OsWRKY45, OsWRKY53, OsWRKY62, OsWRKY55 and OsWRKY71) and their over-expression was shown to confer enhanced resistance to blast infection (Bagnaresi et al., 2012). In plants, perception and transduction of environmental stimuli are largely governed by receptor-like kinases (RLKs). The sequencing of the melon genome elucidated that the number of predicted R-genes in melon was lower than in other plant species (Garcia-Mas et al., 2012). Furthermore, 290 genes were classified as transmembrane receptors, including 161 receptor-like kinases (RLK), 19 kinases containing an additional antifungal protein ginkbilobin-2 domain (RLK-GNK2), and 110 receptor-like proteins (Garcia-Mas et al., 2012). In cucumber and papaya, 61 and 55 genes from the cytoplasmic class were annotated, respectively, in contrast to 212 in Arabidopsis and 302 in grape. These data suggest that the number of NBS–LRR genes is not conserved among plant species and that the value is rather low in *Cucumis*. A plant-specific visualization tool, MapMan, was used to show an overview of the differentially expressed RLKs in the healthy resistant plant in respect of CHT (NAD vs CHT) at the two time points (Figure 13). A *wall associated kinase* (WAK) *gene* (MELO3C008436) was expressed at 24 hpi only in the resistant plant (Table 3), while two genes seemed constitutively induced during the experiment (Figure 13). In *Arabidopsis*, WAKs are involved in cell expansion, pathogen resistance and heavy metal stress tolerance (Li et al., 2009; Yang et al., 2012).The AtWAK1 potentially acts as a receptor of oligogalacturonides elicitors (Brutus et al., 2010). Eight and three *LRR kinases genes* were up-regulated in NAD at 24 and 48 hpi, respectively. Two *Domain of Unknown Function (DUF26) RLKs genes* were recovered in NAD only at 24 hpi (Figure 13). The DUF26 RLKs, also called Cysteine-rich Receptor-like Kinases (CRKs), are transcriptionally induced by oxidative stress, pathogen attack and application of SA and have been suggested to play important roles in the regulation of pathogen defense and programmed cell death (Wrzaczek et al., 2010). Based on RNA-Seq data a *Lectin receptor kinases* and two *cytoplasmic RLK* (RLCKs) *genes* were early activated in NAD and seemed to be constitutively over expressed in NAD in respect of CHT. Lectin are RLKs characterized by an extracellular lectin motif. Plant

lectin receptor kinases are thought to play crucial roles during development and in the adaptive response to various stresses. Although the function of many plant lectin receptors is still not clear, a role for this kinase family in plant innate immunity is emerging (Bouwmeester et al., 2011; Singh et al., 2013). Some well-characterized RLCKs include tomato Pto (disease resistance), Arabidopsis CDG1 (growth and differentiation), Arabidopsis CRCK1 (abiotic stress response), Arabidopsis PBS1 (disease resistance), and wheatgrass Esi47, stress responsive gene involved in hormone signaling (Muto et al., 2004; Yang et al., 2004). Taken together these results demonstrate that NAD, instead of CHT, is more ready and capable of expressing a baseline repertoire of receptors that might be involved in helping a rapid pathogen recognition and then trigger a quick and efficient defense response.

Table 3. List of selected melon transcripts identified in the comparison between the mock-inoculated tissues of NAD *vs* CHT

ID MELONOMICS v3.5	ANNOTATION	log2 Fold Change	
		NAD/CHT	NAD/CHT
		24 hpi	48 hpi
MELO3C009199	duf246 domain-containing protein at1g04910-like	1,6358	-
MELO3C006195	lrr receptor	5,4393	-
MELO3C013983	lrr receptor	3,3953	-
MELO3C023665	lrr receptor	6,2974	-
MELO3C008436	wall-associated receptor kinase 2-like	Inf	7,3445
MELO3C008452	wall-associated receptor kinase 2-like	3,6742	3,1315
MELO3C008453	wall-associated receptor kinase 5-like	3,4295	3,1556
MELO3C015910	wrky transcription factor	1,4534	-
MELO3C006036	wrky transcription factor 30	3,1963	-
MELO3C006725	probable wrky transcription factor 48-like	2,9937	-
MELO3C024135	probable wrky transcription factor 53-like	3,1164	-
MELO3C026932	probable wrky transcription factor 53-like	4,2452	-
MELO3C009383	thaumatin-like protein	-	1,7090
Inf: gene was not expressed in CHT at the same time point			
- Not differentially expressed genes.			
Note: The numbers indicate log2fold change of differentially expressed genes.			

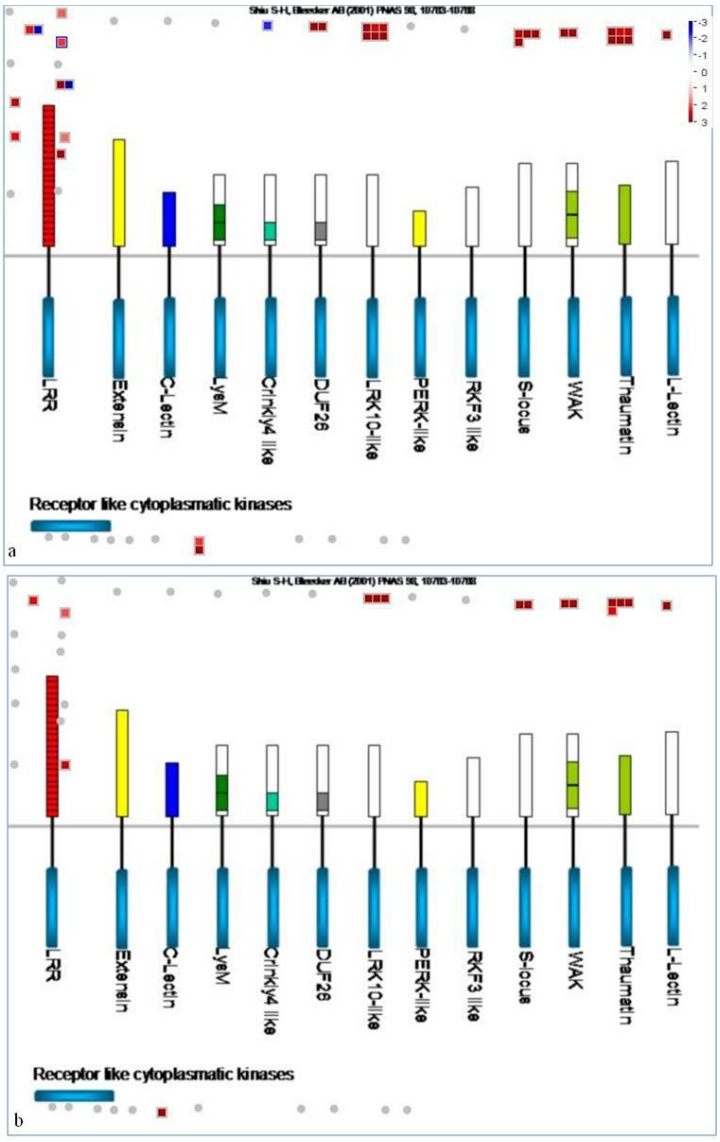


Figure 13. Overview of the RLKs repertoire by comparing mock-inoculated resistant vs susceptible melon plants at 24 hpi (a) and 48 hpi (b). MapMan software (Receptor like kinases_panel) was used to provide a snapshot of modulated genes over the main metabolic pathways. DEGs were binned to MapMan functional categories and log2-fc values are represented. Up-regulated and down-regulated transcripts are shown in red and blue, respectively.

4.7 Analysis of DEGs in the incompatible interaction

4.7.1 Melon defense after FOM1.2 pathogen attack at the initial infection stage

Melon resistance mechanism towards FOM1.2 is still unclear due to its genetic complexity. The expression patterns of genes critically involved in conferring resistance towards this dreadful pathogen revealed a promptly activation in the resistant plant NAD, as reported in other host-pathogen interactions (Bagnaresi et al., 2012; Wang et al., 2014; Sebastiani et al., 2014; Zouari et al., 2014). A number of genes responsive to *F. oxysporum* infection identified in this study have been previously shown to be part of the defense network in various plant–pathogen interactions (Bednarek et al., 2009; Clay et al., 2009; Kidd et al., 2011). Often these transcripts are induced in melon during the incompatible interaction at 24 hpi to ensure specific resistance as well as in the compatible interaction at 48 hpi to centrally contribute to basal resistance, but the timing and abundance differentiate resistance and susceptibility. As soon as the pathogen encounters the plant surface, interaction between the two organisms begins; the pathogen is recognized by the host and the defense signaling networks are activated. Although FOM1.2 infection process is common to *Fusarium oxysporum* species, the mechanism underlying host defense maybe variable (Zhu et al., 2012). Both the constitutive and inducible defense responses contribute to reduced FOM1.2 vascular colonization of melon resistant genotype (Zvirin et al., 2010). In Table 4 are reported selected melon DEGs sustaining the FOM1.2 resistance. The fungal-plant interplay depends on mutual recognition, signaling, the expression of pathogenicity and virulence factors from the fungal side, and the existence of passive, preformed, or inducible defense mechanisms in the plant, resulting in compatible (susceptibility) or incompatible (non host, basal or host specific resistance) interactions (Gonzalez-Fernandez et al., 2010). Specialized pathogenicity genes (R-genes) are directly involved in host-pathogen interactions as reported in tomato (Ma et al., 2013). The number of R-genes in melon was found to be significantly lower than in other species; 411 putative disease resistance R-genes were identified in the melon genome; of these, 81 may exert their disease resistance function as cytoplasmatic proteins through canonical resistance domains, such as the NBS, the LRR, and the TIR domains (Garcia-Mas et al., 2012). Most of the DEGs related to PTI as LRR-receptor family members and PRs exhibited different expression patterns and levels in the resistant DH line NAD (Table 4). Among *10 LRR-receptor DEGs*, 7 were up-regulated only in NAD at 24 hpi and the MELO3C023962 gene showed the highest log2-fc (2.55). To facilitate the inspection of how melon and FOM1.2 networks are overlapping and interacting, expressed genes that code for annotated enzymes were mapped to Kyoto Encyclopedia of Genes and Genomes (KEGG) (Figure 14). KEGG analysis revealed that the unigenes were significantly enriched in various relevant resistance metabolic or signaling pathways. The results obtained other than depict a possible defense response mechanism may offer a platform for further investigations on biological functions of candidate genes in melon responses to FOM1.2. Rapid recognition of a potential invader is a prerequisite for the initiation of an efficient defense response. In the melon-FOM1.2 interaction the chitin elicitor-binding protein (CEPB) and the chitin elicitor receptor kinase (CERK), LysM receptor-like kinases, are important components of the plant signaling cascades that can efficiently transduce input signals into suitable outputs as also reported in banana (Li et al. 2012; Wang et al., 2014). The pathogen chitin recognition (LysM domain-containing GPI-anchored protein 1 and 2-like, MELO3C010601 and MELO3C013264) leads the plant to physical strength the cell wall in order to defend itself against fungal infection. The melon-FOM1.2 interaction followed the model plant immune response signal transduction pathway based on the Ca^{2+} signaling in which signatures of increased cytosolic calcium represent

an essential early event during pathogen response. Ca^{2+} signaling is essential in both plant PTI and ETI responses (Gao et al., 2014) suggesting its role either as second messenger in the activation of resistance responses or as downstream mediator of later cell death acceleration and completion of the defense reaction (Nemchinov et al., 2008). Plant cyclic nucleotide gated ion channels (CNGCs) provide a pathway for Ca^{2+} conductance across the plasma membrane and facilitate cytosolic Ca^{2+} elevation in response to pathogen signals (Ma and Berkowitz, 2011), which leads to downstream generation of pivotal signaling molecules such as NO and ROS. The Ca^{2+} sensed signals are translated into intracellular responses and complex downstream responses, mainly *via* different two types of Ca^{2+} sensor proteins, one of which relays calcium signals as calmodulin (CaM), while the other constitute sensor protein kinases, such as calcium-dependent protein kinases (CDPKs) (Gao et al., 2014). In NAD, the genes coding for CaM and calcium related proteins were highly expressed already at 24 hpi while in CHT were up-regulated (or repressed) only at 48 hpi (Table 4). This behaviour might be ascribed to the lack of effective pathogen sensing mechanisms in CHT, determining a delay in the defense response that lead to the disease and implies the calcium signaling pathway as the key pathway for FOM1.2 resistance in melon. Furthermore, the KEGG map depicts a different situation regarding other signaling molecules that are represented by ROS for the susceptible plant and by the NO for the resistant one. Two major sources of ROS are plasma membrane NADPH oxidase and cell wall peroxidases (PRXs), but how *F. oxysporum*-induced ROS acts in basal resistance and in the HR remain unclear (Almagro et al., 2009, Lehtonen et al., 2011, Zhu et al., 2013). In melon, PRXs represent an important and abundant gene family promptly induced by FOM1.2 infection. Different classes of PRXs were recovered and some of them resulted to be genotype specific, suggesting that different proteins activate distinct transduction pathways that could provoke or the compatible or incompatible interaction. Among the 4 *peroxidases 5-like genes* recovered, 3 DEGs (MELO3C000772, MELO3C025681, MELO3C014652) were specifically regulated in CHT. These data indicate that the oxidative burst process is not only implicated in the resistance, but it is responsible for the susceptibility status in CHT. Among the 5 unique peroxidases sequences regulated in NAD at 24 hpi, two *peroxidases 64* (MELO3C007935 and MELO3C011348) and the *peroxidase 31* (MELO3C021513) genes had the higher log2-fc (>2), supporting their involvement in the resistance (Table 4). The deep oxidative stress status of CHT at 48 hpi, due to the infection, is also demonstrated by the great number (23) of *glutathione transferase related genes* necessary for plant detoxification. While NAD to detoxify itself modulates over time the *glutathione transferase gene* MELO3C016034 (log2-fc from 4.74 to 3.51). These results indicate that CHT triggers significant changes in the antioxidant system leading to a collapse of the protective mechanism at advanced stage of infection. CHT might mimic NAD reaction at 48hpi, but either the lack of FOM1.2 perception or the induction of different gene isoforms, prevent the occurrence of a quick and efficient defense response. However, ROS signaling pathways lead to the HR and cell wall reinforcement. Four genes coding for *subtilisin-like protease* were exclusively up-regulated in NAD at 24 hpi (Table 4). In plant, these genes show some caspase-like activities and were reasonably involved in the outcome of PCD, a response associated with defence commonly referred to as the HR (Vartapetian et al., 2011). The cell wall is the first line of active defense being involved in signal perception from the outside of the plant. It has been suggested that cell wall structural and chemical modifications, elicited by fungi, lead to a directly apposition of substances onto the inner surface that interfere with the invading pathogens (Xin et al., 2012). The up-regulation of PR1 genes indicates that the degradation of the cell wall components (glucans, chitin and proteins) of pathogens is an important defense reaction in melon against FOM1.2 at the initial infection stage (Figure 14). NAD reacts to FOM1.2 penetration by regulating many genes responsible for reinforcing cell wall such as 8 *genes encoding hydroxyproline- rich glycoprotein* (HRGP), 6 for *proline-rich glycoprotein*, 1 for *cellulose synthase*, 2 for *syntaxin* (Table 4). An

analogous behaviour was observed in wheat infected by strip rust and powdery mildew infection (Zhang et al., 2014). The defensive surface structures consist mainly of callose, phenolics, lignin, cellulose, pectin, suberin, lipids, hydroxyproline-rich glycoproteins (HRGPs) and PRX that fortify cell wall and isolate the pathogen from healthy host tissue. The increased expression of positive cell wall related proteins might reinforce melon resistance to FOM1.2, while negative protein would play an opposite role as observed in CHT whose different regulation of the same genes is probably responsible for a different reaction to disease (Table 4). Figure 15 shows an overview of the major expression changes underlining the early response (24 hpi) to FOM 1.2 infection in the resistant (Figure 15a) and susceptible (Figure 15b) plants as annotated by MapMan software. Cell wall degrading enzymes (CWDEs) enable a pathogen to invade plant tissues, but on the other hand, their activity may trigger plant defense responses. For example, even low PG activity might be sufficient for degrading homogalacturonan, the main component of pectin, resulting in oligogalacturonides (OGs) release, that are able to elicit defense responses, including accumulation of ROS and PR proteins, and protect plants against pathogen infections (Ferrari et al., 2013). Our data showed a strong polygalacturonase, pectinesterase (PME) and pectin-degrading (pectate lyase) activity involved in the disruption of the cell wall and playing a role in the response to pathogen attack. Among 10 *polygalacturonase genes* recovered, 3 were up-regulated in NAD. The MELO3C009970 remains constantly activated during the time course experiment (log2-fc>2) in NAD, while is up-regulated in CHT only at 48 hpi (log2-fc=1.88) (Table 4). Unexpected result is the recovery of just one *PGIP gene* (MELO3C016384) which shows a similar expression value (log2-fc~1.90) in NAD and CHT at 24 hpi and 48 hpi respectively (Table 4). This finding confirmed that timing represents, more than the fold change, the essential factor for successfully reacting to FOM1.2 invasion. PME activity is regulated by specific inhibitors (PMEI) that may be expressed in plants as a response to pathogens. In NAD at 24 hpi, 5 *pectinesterase inhibitor* and 3 *pectinesterase genes*, probably PME+PMEI forming complex, are specifically co-expressed during pathogenesis; simultaneously in CHT a different one *pectinesterase gene* was expressed (Table 4). Only the *pectinesterase 2-like gene* (MELO3C006208) was regulated over time, with a decreased expression level at 48 hpi (Table 4). Furthermore, the *pectinesterase 53-like gene* (MELO3C015419) was strongly activated just at 48 hpi (log2-fc=4.31). PMEI1 promoter activation is suggested to be a critical molecular event for host defense response and ethylene- and methyljasmonate (MeJA)-mediated *CaPMEI1 gene* expression in pepper (An et al., 2008). These results prove that an efficient resistance response could depend both on time specific events and on the amount of the synergically regulated genes. Some of the proteins and secondary metabolites that accumulate in the xylem sap during fungal colonization include proteases, PR-1, PR-2, PR-3, PR-4, PR-5, PR-9, xyloglucan-endotransglycosylase (XET) and xyloglucan-specific endoglucanase inhibitor protein (XEGIP), phenols, phytoalexins and lignin-like compounds (Yadeta and Thomma, 2013). Among proteases, two DEGs coding for *xylem serine proteinase 1* were induced only in NAD at 24 hpi (Table 4). The β-1, 3-glucanases (PR-2), chitinases (PR3), thaumatin-like proteins (TLP) (PR5) and peroxidases (PR-9) were among the proteins abundantly accumulated in xylem sap during incompatible interaction at early time point, implying that the presence of these proteins could inhibit or slack the growth of FOM1.2 in melon xylem vessels (Table 4). Upon inoculation β-glucanases activity increased more rapidly in NAD than in CHT. For instance, significant and simultaneous up-regulation of 4 *PR2 genes* (MELO3C006572, MELO3C002353, MELO3C008769, MELO3C013770) listed in GO group "cellulase activity" (GO:0008810) was observed in NAD, while in CHT just one down-regulated *PR2 gene* (MELO3C026609) was recovered. In melon, chitinase activity, elicited by chitin oligosaccharides, have long been implicated in defense responses against invading pests and pathogens (Li et al 2012). The "chitinase activity" (GO:0004568), "chitin catabolic process" (GO:0006032), "response to chitin" (GO:0010200), "endochitinase activity" (GO:0008843) and "chitin binding" (GO:0008061) were

the identified enriched GO terms in melon-FOM1.2 pathosystem. Induced chitinases appear to belong to distinct classes, probably reflecting that efficient chitinase activity may require a pool of various chitinase classes acting in concert. Eleven and seven *PR3 chitinases genes* were induced by FOM1.2 infection in NAD at 24 and 48 hpi respectively, of which MELO3C017677, MELO3C006704, MELO3C026772 sequences were reputed to exert effective defense roles against FOM1.2 since they were expressed only at the first time point (Table 4). There is a relationship between chitinase III and melon resistance to FOM (Balde et al., 2006; Narayanasamy, 2008). Of note, the MELO3C010248 DEG showed an increasing expression level from 24 to 48 hpi in NAD (log2-fc=2.70 and 3.65 respectively), but it was recovered only at 48 hpi in CHT (log2-fc=2.35). Antimicrobial activity of PR5 proteins has been demonstrated towards multiple pathogens (Van Loon et al., 2006). Constitutive expression of TLPs is typically absent in healthy plants and they were induced in response to pathogen attack, probably acting by modifying the permeability of fungal membrane (Acharya et al., 2013). A total of 10 *TLPs genes* was discovered in melon-FOM1.2 interaction, of which 5 were unique in NAD at 24 hpi (log2-fc>1.75) (Table 4). The MELO3C005642 gene deserved particular attention because was always expressed in both genotypes, but it exerted a dramatic up-regulation in the resistant plant during the time course experiment (log2-fc from 7.30 to Infinite) (Table 4). These results are in agreement with the one reported by Rubio et al. (2014) which observed a clear expression of thaumatin-like protein genes in the sample showing sharka symptoms after Pox plum virus (PPV) inoculation. Furthermore, several DEGs had an opposite regulation between the two genotypes showing late and/or missing response in CHT, as observed for other three *PR* MELO3C013762, MELO023694 and MELO3C010919 and for the *chalcone synthase* (CHS) *genes*. The CHS expression, induced in response to phytopathogens, elicitors or wounding in different parts of the plant, resulting in enhanced production of flavonoids (Dao et al., 2011). Consistent with the probability that all plants contain at least one CHS gene (Dao et al., 2011), in NAD the only *CHS gene* (MELO3C014767) recovered was induced at 24 hpi (log2-fc=2.13) (Table 4). On the contrary, the unique CHS sequence (MELO3C010520) in CHT was greatly down-regulated (log2-fc=-6.23), confirming the role of the CHS in protecting plants against biotic stress. Another gene family involved in the innate immune system primarily directed against fungal pathogens is represented by defensins and thionins, that are small, highly stable, cysteine-rich peptides, as identified in pea, tobacco, Arabidopsis and spruce (Stec, 2006; Stotz et al., 2009). The *defensin J1-2-like gene* (MELO3C005214) was only activated in NAD at 24 hpi (log2-fc=1.33), but it showed an opposite regulation in CHT, repressed at 24 hpi (log2-fc=-1.77) and more induced at 48 hpi (log2-fc=2.66). The *thionin-like protein 2 gene* (MELO3C023361) displayed similar regulation in both early (log2-fc=2.21) and late (log2-fc=1.69) NAD infection, while it was up-regulated only at 48 hpi with a higher expression value (log2-fc=4.24) in CHT (Table 4). Nevertheless these DEGs were greatly induced by the susceptible plant, the repressed/missed response at 24 hpi bring to the disease, accentuating the importance of a prompt reaction.

4.7.2 Signal transduction networks in melon-FOM1.2 resistance

The signatures of increased cytosolic Ca^{2+} levels triggered by the fungus infection and sensed by specific calcium proteins lead to the subsequent activation of cellular responses, including transcriptional reprogramming, activation of the phosphorylation cascade, and accumulation of secondary metabolites (Gao et al., 2014). Defense-associated genes are normally regulated positively or negatively by transcription factors (TF) that are direct or indirect targets of various

signal transduction pathways. Many TF families have been shown to play important roles in defence responses through regulating the expression of defence-related genes. In some plant MYB proteins are involved in defence responses to pathogens. MYB proteins perform diverse biological functions in the cell cycle and in development, in the regulation of primary and secondary metabolism, and abiotic stress response (Liu et al., 2013). For instance, *Arabidopsis* R2R3-MYB proteins, including AtMYB108 and AtMYB96, participate in disease resistance (Mengiste et al., 2003; Seo and Park, 2010). Eight *MYB TF genes* were up-regulated only in NAD (Table 4). In the resistant plant the perception of chitin elicitor induces the activation of the mitogen-activated protein kinase (MAPK) cascade and WRKY family TFs as also reported in *Arabidopsis* (Reddy, et al., 2011; Popescu et al., 2007) (Figure 14). KEGG Map (Figure 14) suggestes that PTI in melon-FOM1.2 interaction was activated through ROS and NO signaling pathways mediated by CDPK, RBOH and CaM/CML, MKK4/5 and WRKY25/33 involved a MAPK cascade, while ETI was triggered via RIN4, RPM1,PBS1, RAR1 (required for Mla12 resistance), SGT1 (suppressor of the G2 allele of SKP1) and heat shock protein (HSP90). Among the genes expressed in the ETI response, only the HSP90 resulted up-regulated (differentially expressed) in CHT at 48 hpi. In addition, MIN7 (guanine nucleotide-exchange factor) and JAZ (jasmonate ZIM domain-containing protein) together with MYC2-like were showed to activate protein-mediated proteolysis and the defense-related *gene PR1*. In the susceptible plant, *COI1* and *MYC2* genes were repressed at 48hpi (Figure 14). An analogous behaviour was observed in wheat infected by strip rust and powdery mildew infection (Zhang et al., 2014). These downstream regulation was only found in the resistant plant NAD that up-regulated the MAPKK4 and MAPKK5 (MELO3C002150 and MELO3C025790) and 4 *WRKY genes* at 24 hpi and one *WRKY6 gene* at 48 hpi (Table 4). Recently, a MAPK cascade involving MKK4 and the MAPKs MPK3 and MPK6 was shown to transduce chitin elicitor signal to defense responses including biosynthesis of diterpenoid phytoalexins in rice (Bagnaresi et al., 2012). In *Arabidopsis* and tobacco the activation of MAPKK4/MAPKK5-MAPKK6 cascade increased ethylene synthesis (Jakubowiczm et al., 2010). As one might expect, plant hormone-signaling pathways also play key roles in plant defense against FOM1.2 (Figure 15). The SA, JA and ET hormone pathways are important regulators of defense-gene expression (Bari and Jones, 2009). Thus, the successful outcome of a given response depends on the interaction among phytohormone signaling pathways rather than on the independent contribution of each of them (Jakubowiczm and Nowak, 2010). The analysis of the SA signaling-related genes showed no significant differences between the two genotypes. Although recent studies showed a complex and ambiguous role of SA against necrotrophic pathogens like *Fusarium oxysporum*, leading to many intriguing questions about its relationship between other signaling compounds (Berrocal-Lobo and Molina 2007; Swarupa et al., 2014), in this work a major number of genes related to the SA pathway was depicted in CHT at 48 hpi, probably suggesting the not involvement of SA in the resistance to FOM1.2. Only 7 *DEGs* coding for novel *protein containing ankyrin repeats* showed a high induction in NAD in response to FOM1.2 (Table 4). The ankyrin repeat is a motif containing 33 amino acids involved in protein-protein interactions (Sedgwick and Smerdon, 1999). In Arabidopsis ACD6, an ankyrin containing protein, is a regulator and an effector of SA signaling defense response (Lu et al., 2003). Nevertheless, FOM1.2 resistance seems mainly mediated by JA and ET pathways as observed also in banana (Li et al., 2012). The role of JA signaling in plant defense against necrotrophic pathogens is well recognized. During infection, NAD induced, mainly at 48 hpi, several JA-dependent defense genes such as *germin-like* and *PR4* and JA biosynthesis enzymes as *lipoxygenase* (LOX) (Table 4). DEGs coding for ET responsive factors were among the most numerous induced transcripts in NAD at 24 hpi (Table 4), as also suggested by the MapMan analysis (Figure 15). Furthermore, the ET signaling genes (i.e. CRF, LEP, RAP, TINY, ERF) resulted early up-regulated in NAD and among them the ERF TFs (Table 4) are known to be involved in the regulation of JA-dependent defenses (Lorenzo et

al., 2003; Berrocal-Lobo and Molina, 2007). In addition, other hormones such as auxin and ABA, originally described for their function in the regulation of plant growth processes and the response to abiotic stresses, have recently emerged as crucial players in plant–pathogen interactions (Mauch-Mani and Mauch, 2005; Kazan and Manners, 2009; Ton et al., 2009; Fu and Wang, 2011). However, the crosstalk between the so-called "defense hormones" and "growth hormones" during plant defense is still largely unknown. All the phytohormone pathways are linked to each other in a huge, complex and still obscure network. For example, ET, ABA, auxin, gibberellins, and cytokinins pathways are considered as hormone modulators of the SA–JA signaling backbone (Pieterse et al., 2012). Figure 15 shows that auxin, ABA and BRs pathways are greatly involved in the resistance response to FOM1.2. The phytohormone auxin is a key signaling molecule regulating a wide range of growth and developmental processes. In addition, auxin regulates various cellular processes that are associated with the plant response to biotic stresses (Kazan and Manners, 2009). Similar to SA and JA, auxin appears to differentially affect resistance to different pathogen groups. Several studies have shown that pathogen infection results in imbalances in auxin levels as well as changes in the expression of genes involved in auxin signaling (Bari and Jones, 2009). Repression of auxin signaling by the SA pathway was recently shown to contribute to plant resistance to biotrophic pathogens (Navarro et al, 2006; Wang et al., 2007). For instance, auxin signaling is required for resistance to necrotrophic pathogens such as *Botrytis cinerea* and *P. cucumerina* (Llorente et al., 2008) and susceptibility to the bacterial pathogen *Pseudomonas syringae* and the biotrophic oomycete pathogen *Hyaloperonospora parasitica* (Wang et al., 2007). In contrast to the well-recognized antagonistic crosstalk between SA and auxin during plant resistance to biotrophic pathogens, JA and auxin may interact positively in plant resistance to necrotrophic pathogens (Kazan and Manners, 2009; Qi et al., 2012). Although auxin resulted to promote susceptibility to *F. oxysporum* (Kidd et al., 2011), in this work auxin related genes as *auxin influx carrier protein* (MELO3C013367; MELO3C003299), *auxin induced protein 5NG4-like* (MELO3C003783), *auxin responsive family protein X15-like* (MELO3C000885), *auxin responsive family protein 15A-like* (MELO3C005991) and *auxin responsive proteins IAA13-like* (MELO3C023046; MELO3C006371) were exclusively induced in NAD at 24 hpi, suggesting their positive involvement in the resistance mechanism (Table 4). Based on auxin related pathway plays a dominant role in regulating NAD resistance, both NAD and CHT plants, infected with FOM1.2, were treated with exogenous auxin (IAA) applications to investigate the involvement of auxin in regulating plant defense responses or disease development. FOM1.2 infection activates the transcription of a battery of auxin biosynthetic genes and therefore elevates auxin biosynthesis of host plants. Melon plants were daily phenotypically screened to assess the effects of auxin on the development of the disease. In addition to the infection with FOM1.2, the inoculation procedure itself causes further stress either in susceptible or in resistant plants (Figures 16a, b, c). Melon plants (resistant and susceptible) usually begin to lose turgor during the first hours after infection but the pre-treatment with exogenous auxin, performed 48 hours before the fungal inoculation, has allowed a better and quick recovery of all the plants (Figures 16d, e, f, g). As NAD, the susceptible genotype appeared turgid and no disease symptoms were observed. During the 15 dpi, CHT and NAD plants elongated and new shoots and leaves were formed (Figures 16h, i, j, k). Instead, in the compatible interaction (CHT plants not treated with IAA) symptoms as necrosis and severe lesions on plant collars became obvious already before 8 dpi and the majority of plants died until the fifteenth dpi. After 18 dpi, only three plants of CHT were dead (Figure 16l), while at 21 dpi only two plants were still living. Obviously NAD resistant plants turned all alive and phenotypically healthy (Figure 16m). However, the use of two different concentrations of IAA (5 and 10 mM) did not result in visually appreciable improving effects on melon resistance. The results indicate a positive correlation between auxin and an increasing of FOM1.2 tolerance, and probably if a higher concentration of IAA was supplied to CHT, a long-

lasting plant survival could be obtained. Further studies are needed to validate this data. During the infection process, FOM1.2 induces water stress by occluding the xylem vessels. In melon the lack of water supply to the leaves during infection, leads to altered/suppress other processes like photosynthetic rate, stomatal conductance and transpiration rate, as also reported in banana by Dong et al. (2012). This critical status enhanced the biotic stress i.e. pathogen attack and colonization inside the plant and could be correlated with the onset of disease symptoms in CHT. On the contrary, the resistant plant was able to overcome this problem overexpressing 5 *specific receptors genes* implicated in the ABA signaling pathway (Table 4). It was well established that the phytohormone ABA serves as an endogenous messenger in drought stress responses of plants (Raghavendra et al., 2010). Cell expansion can be maintained or decreased, depending on the preserve of turgor and cell wall extensibility regulated by phytohormones like ABA and other local and systemic factors involved in coordination of the drought responses. In response to drought stress, ABA stimulates a signaling pathway that triggers the production of ROS, which in turn induces an increase in cytosolic Ca^{2+} (Osakabe et al., 2014). This data is completely in agreement with those reported in the above section, in which Ca^{2+} was identified as the pivotal intracellular messenger induced by FOM1.2 infection.

Table 4. List of selected melon transcripts regulated after FOM1.2 inoculation

FUNCTIONAL CATEGORY	ID MELONOMICS v3.5	ANNOTATION	log2 Fold Change			
			NAD	NAD	CHT	CHT
			24 hpi	48 hpi	24 hpi	48 hpi
Ca^{+2} RELATED PROTEINS	MELO3C014698	calmodulin	1,18	-	-	-
	MELO3C006721	hypersensitive reaction associated ca2+-binding protein	1,88	-	-	2,35
	MELO3C004465	calmodulin-like protein 4-like [Cucumis sativus]	7,12	-	-	-
	MELO3C015088	calmodulin binding	-	2,18	-	-
	MELO3C014686	calmodulin binding	-	2,03	-	1,69
	MELO3C017117	calmodulin binding protein	-	1,22	-	1,15
	MELO3C004202	calmodulin-binding protein	-	1,98	-	1,91
	MELO3C009591	calmodulin binding	-	-	-	-1,86
PEROXIDASES	MELO3C002457	peroxidase	1,23	-	-	-
	MELO3C007935	peroxidase 64	2,13	-	-	-
	MELO3C011348	peroxidase 64	3,78	-	-	-
	MELO3C014658	peroxidase 2-like	1,15	-	-	-
	MELO3C021513	peroxidase 31	2,25	-	-	-
CELL WALL REINFORCEMENT	MELO3C013954	14 kda proline-rich	2,90	-	-	-
	MELO3C011271	14 kda proline-rich protein	3,42	-1,60	-1,50	-
	MELO3C013952	14 kda proline-rich protein	2,28	-3,64	-3,16	-
	MELO3C023952	proline-rich family expressed	1,58	-	-	-
	MELO3C026077	proline-rich protein	1,99	-	-1,52	-
	MELO3C012194	proline-rich receptor-like protein kinase PERK9-like	-	1,41	-	1,17
	MELO3C007692	hydroxyproline-rich glycoprotein	1,10	-	-	-
	MELO3C025771	hydroxyproline-rich glycoprotein family protein	1,49	-	-	2,06
	MELO3C024371	hydroxyproline-rich glycoprotein family protein	-	2,25	-	1,91
	MELO3C009532	late embryogenesis abundant hydroxyproline-rich glycoprotein	2,42	-	-	-
	MELO3C012699	late embryogenesis abundant hydroxyproline-rich glycoprotein	1,67	-	-	-
	MELO3C014516	late embryogenesis abundant hydroxyproline-rich glycoprotein	2,27	-	-	-
	MELO3C016302	late embryogenesis abundant hydroxyproline-rich glycoprotein	2,54	-	-	-
	MELO3C025270	late embryogenesis abundant hydroxyproline-rich glycoprotein	1,30	-	-	-
	MELO3C022385	cellulose synthase	1,15	-	-	-
	MELO3C009531	syntaxin-24-like	2,32	1,54	-	2,54
	MELO3C009530	syntaxin-24-like	1,63	1,49	-	2,23
SUBTILISIN PROTEASE	MELO3C007609	subtilisin-like protease	1,76	-	-	-
	MELO3C015526	subtilisin-like protease	1,33	-	-	-
	MELO3C023644	subtilisin-like protease-like	1,40	-	-1,22	-
	MELO3C024314	subtilisin-like protease-like	1,26	-	-	-
PGs	MELO3C009970	polygalacturonase at1g48100-like	2,59	2,00	-	1,89
	MELO3C010662	probable polygalacturonase non-catalytic subunit jp650-like	1,60	-	-	1,21
	MELO3C017098	polygalacturonase-1 non-catalytic subunit beta	2,05	-	-	-
	MELO3C016384	polygalacturonase-inhibiting protein	1,87	-	-	1,95
	MELO3C022704	pectin methylesterase	1,94	-	-	-
	MELO3C007375	pectin methylesterase	1,70	-	-	-
	MELO3C006208	pectinesterase 2-like	2,21	1,37	-	2,71
	MELO3C005291	pectinesterase inhibitor	2,79	-	-	-
	MELO3C005885	probable pectinesterase pectinesterase inhibitor 34-like	2,02	-	-	-
	MELO3C015963	probable pectinesterase pectinesterase inhibitor 40-like	1,70	-	-	-
	MELO3C023253	probable pectinesterase pectinesterase inhibitor 51-like	2,62	-	-	1,95
	MELO3C023254	probable pectinesterase pectinesterase inhibitor 51-like	1,80	-	-	1,44
	MELO3C010953	pectinesterase 11-like	-	4,84	-	
	MELO3C002319	pectate lyase	2,57	-	-	-
	MELO3C024477	probable pectate lyase 12-like	3,00	-	-1,33	-
LRR RECEPTORS	MELO3C004602	leucine-rich repeat extensin-like protein 4-like	1,34	-	-	-
	MELO3C007317	f-box lrr-repeat protein	1,45	-	-	1,88
	MELO3C007887	probable lrr receptor-like serine threonine-protein kinase at4g36180	1,70	-	-	-
	MELO3C014415	leucine-rich repeat family protein	1,55	-	-	-
	MELO3C014614	leucine-rich repeat receptor-like protein	1,50	-	-	-
	MELO3C017541	serine-threonine protein plant-	2,35	-	-	-
	MELO3C023962	leucine-rich repeat receptor-like protein	2,55	-1,49	-1,84	-
	MELO3C026359	leucine-rich repeat family protein	2,39	-	-	-
	MELO3C023665	leucine-rich repeat receptor-like tyrosine-protein kinase at2g41820-	-	2,34	-	-
	MELO3C002662	probable lrr receptor-like serine threonine-protein kinase at2g24230	-	1,24	-	-
XYLEM PROTEASE	MELO3C023640	xylem serine proteinase 1-like isoform 1	1,54	-	-	-
	MELO3C024124	xylem serine proteinase 1-like isoform 1	1,27	-	-	-

DEFENSE RELATED PROTEIN	MELO3C015551	FMO1	-	Inf	-	-
	MELO3C014767	chalcone synthase	2,13	-	-	-
	MELO3C007732	E3 Ubiquitin protein ligase ATL6 like	Inf	-	-	-
	MELO3C006572	endo- -beta-d-glucanase-like	1,63	-	-	1,34
	MELO3C023353	endo- -beta-glucanase	1,83	-	-	-
	MELO3C008769	endo- -beta-glucanase	1,45	-	-	1,96
	MELO3C013770	endo- -beta-glucanase	1,26	-	-	3,24
	MELO3C010248	pathogenesis-related protein 3	2,70	3,65	-	2,35
	MELO3C017677	chitinase-like protein 1-like isoform 2	1,55	-	-	-
	MELO3C006704	chitinase-like protein 2	1,36	-	-	-
	MELO3C007962	class i chitinase	2,82	4,62	1,46	2,31
	MELO3C007961	class i chitinase	2,46	4,28	1,86	4,08
	MELO3C007966	class i chitinase	1,45	-	-	1,40
	MELO3C026772	acidic endochitinase-like	1,65	-	-	-
	MELO3C005642	thaumatin-like protein	7,29	Inf	4,61	7,79
	MELO3C009903	thaumatin-like protein	2,96	-	-	-
	MELO3C002128	thaumatin-like protein	2,80	-	-	-
	MELO3C013762	thaumatin-like protein 1-like	2,68	-	-1,59	-
	MELO3C003144	pathogenesis-related thaumatin-like protein	2,46	-	-	-
	MELO3C011018	thaumatin-like protein 1-like	1,75	-	-	-
	MELO3C010919	pathogenesis-related protein 3	2,70	3,65	-	2,35
	MELO3C023694	pathogenesis-related protein pr-1	1,80	-	-2,14	-
	MELO3C005940	pathogenesis-related thaumatin-like protein	2,46	-	-	-
	MELO3C023361	thionin-like protein 2-like	2,21	1,69	-	4,24
	MELO3C005214	defensin J1-2-like [Cucumis sativus]	1,33	-	-1,77	2,66
MAPK	MELO3C002150	mitogen-activated protein kinase kinase 4-like	1,14	-	-	-
	MELO3C025790	map kinase kinase 5	1,25	-	-	-
WRKY TF	MELO3C005937	probable wrky transcription factor 12-like	1,40	-	-	-
	MELO3C007576	probable wrky transcription factor 15-like	2,37	-	-	1,30
	MELO3C016774	dna-binding protein wrky	1,19	-	-	-
	MELO3C016947	dna-binding wrky	2,42	-	-	-
	MELO3C007409	wrky transcription factor 6-like	-	1,26	-	
MYB TF	MELO3C015228	MYB86	2,22	-	-	-2,44
	MELO3C011547	myb-like transcription factor family protein	1,84	-	-	-
	MELO3C010833	transcription factor myb44-like	1,19	-	-	-
	MELO3C010893	transcriptional activator myb-like	2,83	-	-	-
	MELO3C021284	myb transcription factor	-	1,99	-	-
	MELO3C012160	myb-like protein sp	-	-	-2,26	-
	MELO3C015228	myb transcription factor	2,22	-	-	-2,44
	MELO3C009733	myb family transcription factor	1,91	-	-1,56	-
ANCHYRIN REPEAT CONTAINING PROTEINS	MELO3C009784	ankyrin repeat protein rf_0381-like	2,33	-	-	-
	MELO3C009379	ankyrin repeat-containing	2,12	-	-	1,47
	MELO3C011476	ankyrin repeat-containing protein at2g01680-like	1,74	5,24	3,25	2,08
	MELO3C011477	ankyrin	Inf	4,41	2,59	4,37
	MELO3C011475	ankyrin repeat-containing protein at2g01680-like	-	2,65	-	-
	MELO3C002441	ankyrin repeat-containing protein at5g02620-like	-	4,03	-	2,38
	MELO3C013066	ankyrin repeat-containing protein at5g02620-like	-	2,41	-	-
LOX	MELO3C007767	lipoxygenase homology domain-containing protein 1-like	2,00	-	-	1,39
	MELO3C005822	lipoxygenase	2,90	-	-	-
	MELO3C014636	linoleate 13s-lipoxygenase 2- chloroplastic-like	-	4,06	-	-
	MELO3C004244	probable linoleate 9s-lipoxygenase 5-like	-	1,68	-	-
	MELO3C004247	probable linoleate 9s-lipoxygenase 5-like	-	1,63	-	-
AUXIN RELATED GENES	MELO3C013367	auxin influx carrier component	2,43	-	-	-
	MELO3C003299	auxin influx carrier protein	1,29	-	-	-
	MELO3C003783	auxin-induced protein 5ng4-like	1,98	-	-	-
	MELO3C000885	auxin-responsive family protein	1,69	-	-	-
	MELO3C005991	auxin-responsive family protein	1,99	-	-	-
	MELO3C006371	auxin-responsive protein iaa13	1,42	-	-	-
	MELO3C023046	auxin-responsive protein iaa13	1,43	-	-	-

ABA RECEPTORS	MELO3C009136	abscisic acid receptor pyl4-like	2,38	-	-	-
	MELO3C026019	abscisic acid receptor pyl4-like	2,20	-	-	-
	MELO3C002656	abscisic acid receptor pyl4-like	1,73	-	-	-
	MELO3C023724	abscisic acid receptor pyl8	1,13	-	-	-
	MELO3C013854	abscisic acid receptor pyr1-like	1,17	-	-	-
ETHYLENE RESPONSIVE FACTORS	MELO3C002623	ethylene-responsive transcription factor 12-like	4,02	1,51	-	1,64
	MELO3C005088	ap2 erf domain-containing transcription factor	2,31	-	-	-
	MELO3C005465	ethylene-responsive transcription factor 5-like	1,10	-	-	-
	MELO3C005502	ap2 erf domain-containing transcription factor	4,03	-	-	2,39
	MELO3C005734	ap2 erf domain-containing transcription factor	2,70	-	-	-
	MELO3C005972	ethylene-responsive transcription factor erf034-like	3,12	-	-	-
	MELO3C006149	ap2 erf domain-containing transcription factor	2,57	-	-	-
	MELO3C006870	ap2 erf domain-containing transcription factor	1,45	2,05	-	1,95
	MELO3C006973	ethylene-responsive transcription factor crf4-like	1,99	-	-	-
	MELO3C007267	ethylene-responsive transcription factor 4-like	1,49	-	-	-
	MELO3C010840	ap2 erf domain-containing transcription factor	1,67	-	-1,31	-
	MELO3C011286	ap2 erf domain-containing transcription factor	1,91	-	-	-
	MELO3C011287	ap2 erf domain-containing transcription factor	3,12	-	-	1,69
	MELO3C011671	ap2 erf domain-containing transcription factor	2,69	-	-	2,35
	MELO3C013916	ap2 erf domain-containing transcription factor	1,90	-	-	1,71
	MELO3C013917	ethylene-responsive transcription factor 5-like	1,55	-	-	-
	MELO3C014181	ethylene-responsive transcription factor 3-like	1,73	-	-	-
	MELO3C014441	ethylene-responsive element binding factor-like protein	1,67	-	-	-
	MELO3C016285	ethylene-responsive transcription factor erf061-like	1,56	-	-	-
	MELO3C016780	ap2 erf domain-containing transcription factor	1,83	-	-	1,25
	MELO3C017860	ap2 erf domain-containing transcription factor	2,86	-	-	1,62
	MELO3C017940	ap2 erf domain-containing transcription factor	2,49	1,33	-	1,66
	MELO3C021306	ethylene-responsive transcription factor rap2-3-like	2,59	-	-1,70	1,88
	MELO3C022281	ethylene-responsive transcription factor erf034-like	1,95	-	-	-
	MELO3C022718	ethylene-responsive transcription factor-like protein	2,11	-	-	-
	MELO3C022718	ethylene-responsive transcription factor-like protein	2,11	-	-	-
	MELO3C025608	ethylene-responsive transcription factor 4-like	1,74	-	-	-
	MELO3C006431	ap2 erf domain-containing transcription factor	-	2,56	-	-4,66
	MELO3C010425	ap2 erf domain-containing transcription factor	-	2,10	-	1,45
	MELO3C015961	ethylene-responsive transcription factor 1b-like	-	2,48	-	-

Inf: the gene was not expressed in the mock-inoculated plant at the same time point

- Not differentially expressed genes.

Note: The numbers indicate log2fold change of differentially expressed genes.

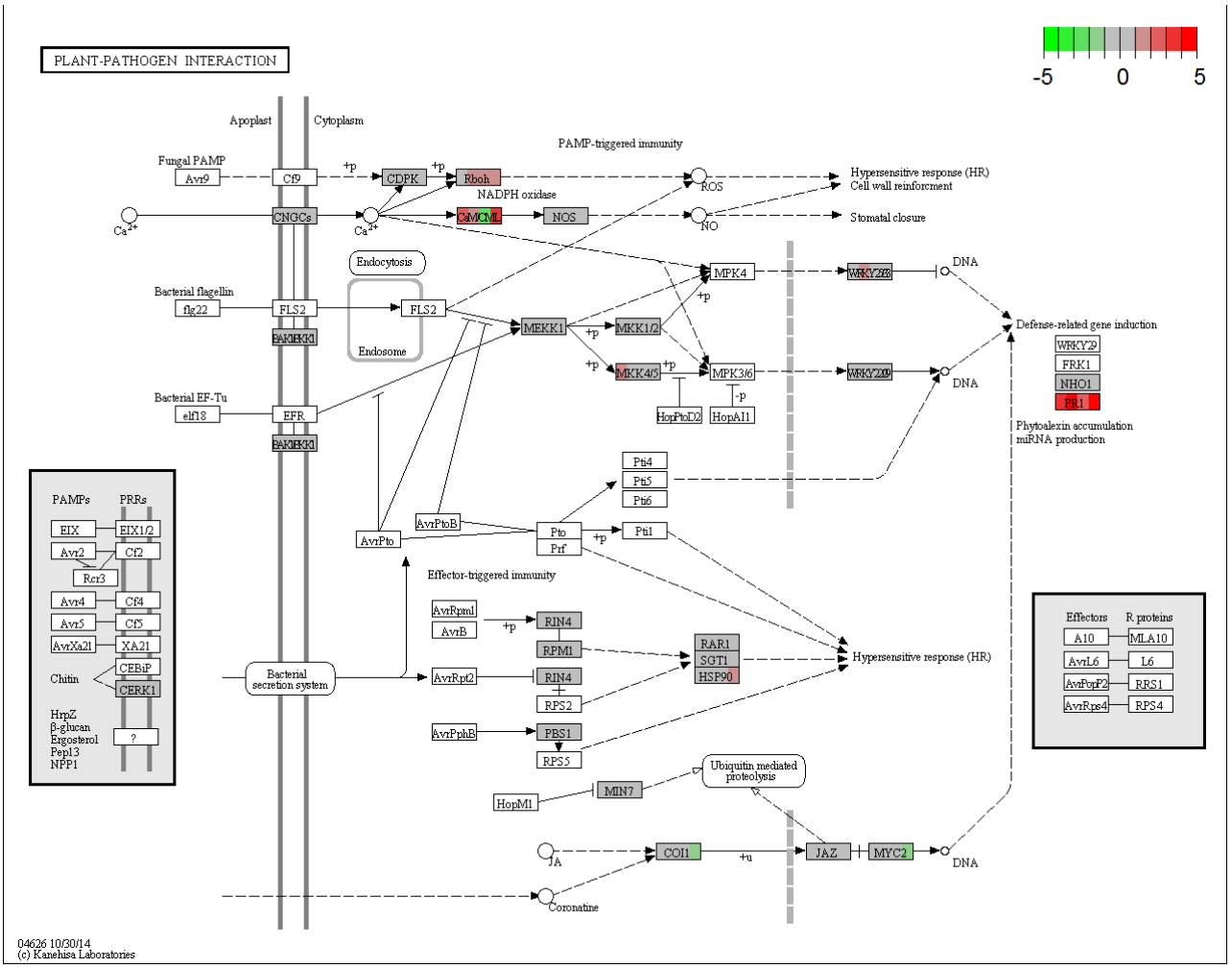


Figure 14. KEGG orthology map of melon plant-pathogen interaction (ko04626). Coloured boxes are placeholders for one or more genes as assigned to the same KEGG Orthology (KO) group by KAAS server. For each box, from left to right, contrasts NAD24infected_vs_NAD24control, NAD48infected_vs_NAD48control, CHT24infected_vs_CHT24control and CHT24 infected_vs_CHT48control are represented in color scale representing log2-fc of expression values (inoculated over mock) as indicated in color key, from -5 (sharp green) to +5 (sharp red).

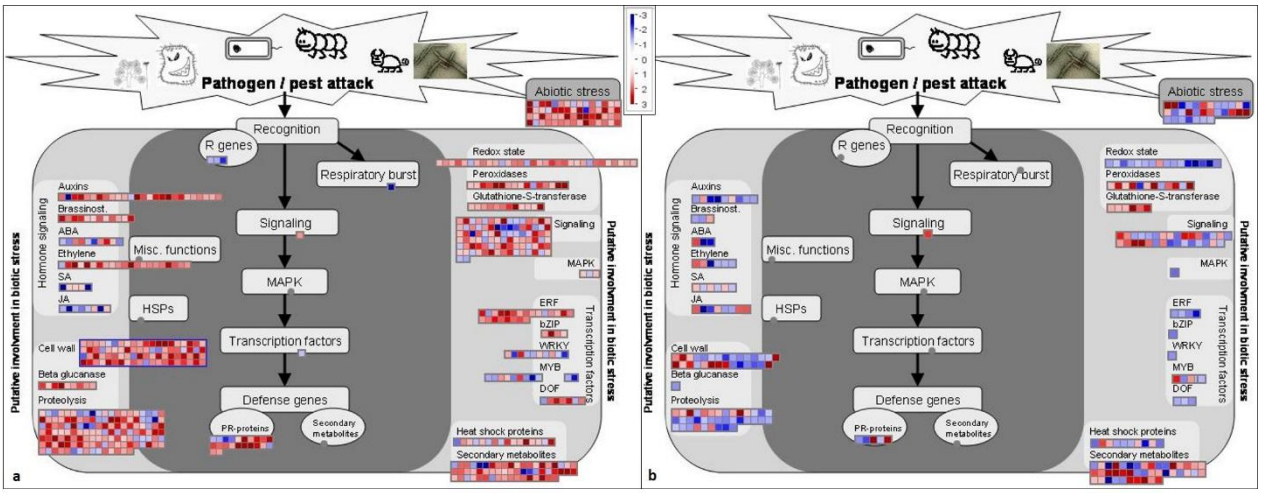


Figure 15. Overview of the effect of FOM1.2-induced expression changes on metabolism of resistant (a) and susceptible (b) melon plants at 24 hpi. MapMan software (Biotic stress_panel) was used to provide a snapshot of modulated genes over the main metabolic pathways. DEGs were binned to MapMan functional categories and log2-fc values are represented. Up-regulated and down-regulated transcripts are shown in red and blue, respectively.

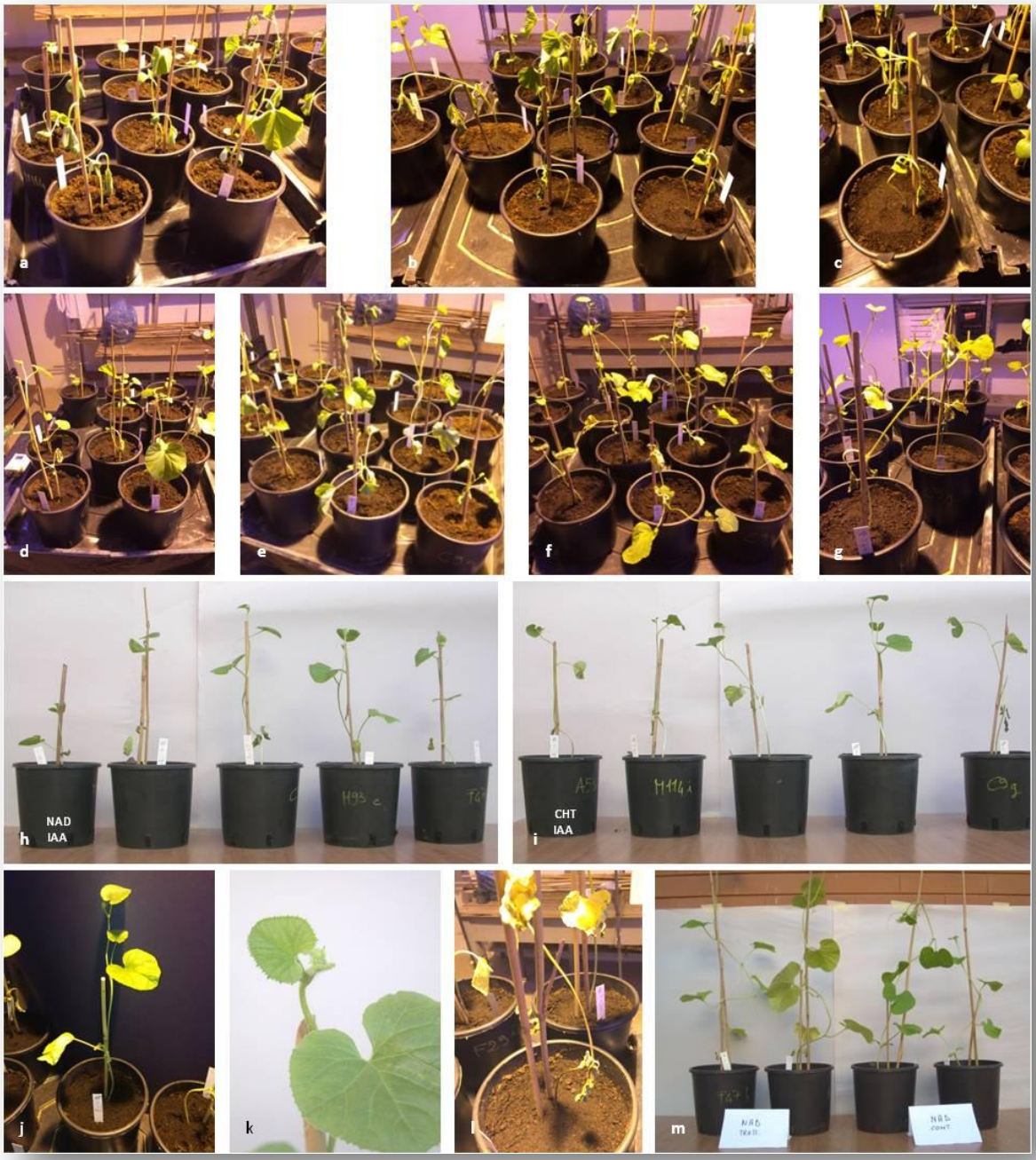


Figure 16. Phenotypic screening of infected melon plants treated with exogenous IAA. The inoculation procedure with FOM1.2 itself causes water stress either in resistant (a) or in susceptible (b, c) melon plants. The pre-treatment with exogenous auxin has allowed a better and quick turgor recovery (just at 24 hpi) of resistant (d) as well as susceptible (e, f, g) plants. During the 15 dpi, both NAD (h) and CHT (i, j) appeared visible turgid and no disease symptoms were observed; plants elongated and new shoots and leaves were formed. k) A detail of apex of infected (IAA treated) CHT at 15 dpi. l) After 18 dpi, CHT began to show disease symptoms. m) Mock-inoculated and infected NAD plants appeared phenotypically healthy at 21 dpi.

4.8 Unannoted DEGs sustaining the FOM1.2 resistance

Several melon *unannotated transcripts* (NA) were found to be differentially regulated after FOM1.2 infection. Although the function of these sequences has been described as "unknown", they could be strongly involved in the disease response. Of the 6401 total DEGs that were assembled from all the genotypes and time points, 1486 (23.2%) were considered novel if they were outside of RefSeq (MELO v3.5) gene annotations. Novel transcripts could include Ensembl Plant transcripts (uncharacterized, hypothetical and predicted protein) or transcripts without prior annotation (unannotated). The patterns of transcript accumulation for these "unknown" sequences were largely in agreement with that of "known" genes and reflected the performance of the two different types of interaction. In particular, in CHT 254 and 542 NA DEGs were identified at 24 and 48 hpi, respectively, while in the resistant plant NAD 511 and 179 NA DEGs were recovered at 24 and 48 hpi, respectively. Great prominence was given to NAD-specific DEGs for highlighting new putative candidate resistance genes. A total of 185 NA DEGs resulted specifically regulated in the resistant plant at 24 hpi, of which 18 were up-regulated with a $\log_2$-fc>3 (Table 5). Among them three sequences were strongly induced at 24 hpi ($\log_2$-fc>2) and were not identified either in CHT or in the mock-inoculated samples (Table 5). These sequences were selected for real time validation since they could be considered as novel candidate resistance genes towards FOM1.2 and could be an important starting point for further functional studies. NAD showed 6 and 14 NA down-regulated genes with a $\log_2$-fc=<-2 at 24 and 48 hpi, respectively (Table 5). Despite the fungus colonizes the vascular tissue of resistant plant (Ficcadenti et al., 2002; Sestili et al., 2011), we suppose that NAD is able to overcome the infection and create a fungal inhospitable environment by repressing these genes, probably involved in enhancing the virulence of the pathogen. Ten DEGs were identified in NAD at both time points with a trend of transcript accumulation up and down-regulated at 24 and 48 hpi respectively; only 2 sequences showed the opposite behaviour (Table 5). The unknown sequence MELO3C018930 was constantly repressed. These data prove that much remains to be discovered about the molecular events associated with FOM1.2 infection in melon.

Table 5. Selected unannotated DEGs sustaining NAD resistance to FOM1.2

UP - NA DEGs 24 hpi-SPECIFIC (log2fold change >2)	MELO3C002948, MELO3C010155, MELO3C015129
NA DEGs UP-REGULATED AT 24 hpi (log2fold change >3)	MELO3C000102, MELO3C007553, MELO3C008379, MELO3C009658, MELO3C016886, MELO3C018504, MELO3C018743, MELO3C018921, MELO3C019103, MELO3C020264, MELO3C020268, MELO3C020660, MELO3C020803, MELO3C022595, MELO3C022838, MELO3C025922, MELO3C027227
NA DEGs DOWN-REGULATED AT 24 hpi (log2fold change <-2)	MELO3C018601, MELO3C018650, MELO3C018794, MELO3C019660, MELO3C020613, MELO3C026880
NA DEGs DOWN-REGULATED AT 48 hpi (log2fold change <-2)	MELO3C007169, MELO3C0115896, MELO3C018472, MELO3C018703, MELO3C018739, MELO3C018845, MELO3C019171, MELO3C019172, MELO3C019239, MELO3C020761, MELO3C020763, MELO3C020766, MELO3C020771, MELO3C024480
NA DEGs DOWN-REGULATED AT 24 hpi AND UP-REGULATED AT 48 hpi	MELO3C018728, MELO3C023628
NA DEGs UP-REGULATED AT 24 hpi AND DOWN-REGULATED AT 48 hpi	MELO3C018470, MELO3C018532, MELO3C020005, MELO3C020396, MELO3C020626, MELO3C020751, MELO3C020752

4.9 Identification of FOM1.2 DEGs in melon during infection

A comprehensive understanding of host–pathogen interactions requires a knowledge of the associated gene expression changes in both the pathogen and the host. Discrimination between the host and the pathogen in the mixed RNA-Seq collections is valuable for dissecting the transcriptome of the infected tissues, for understanding the mechanism of host–pathogen interactions and for developing strategies of fungal disease control (Zhu et al., 2013). However, there are few published studies about the discrimination between plant and fungus origin of RNA-Seq data from plant tissues infected with plant pathogen (Zhu et al., 2013). Fungal sequences expressed *in planta* were analyzed since the fungus has been reisolated from infected melon stems already at 24 hpi in both compatible and incompatible interactions (Sestili et al., 2011). FOM genomics data available up to now are very scarce. Therefore, *Fusarium oxysporum* f.sp. *lycopersici* (Fol) (version F02.20, obtained from http://fungi.ensembl.org/Fusarium_oxysporum/Info/Index) was used as reference genome to detect fungal reads that could be attributable to transcripts related to virulence factors. Toward this end, the contaminant-free reads from all samples as obtained for analysis of melon transcripts were mapped with tophat2 (Kim et al., 2013) against Fol genome and read counts were obtained from BAM alignment files by counting with HTSeq software using the corresponding GTF file as obtained from http://fungi.ensembl.org/Fusarium_oxysporum. DEGs were called via the R package DESeq2 with FDR set to 0.05. The RNA-Seq data were also analyzed to search for orthologous sequences of 100 known pathogenic genes, available in the *Fusarium graminearum* database (http://csb.tongji.edu.cn/efg/search/pathogenic.py). FOM1.2 utilizes different effectors to trigger the plant response since the virulence factors have not been yet identified. All Fusarium genomes encode a suite of cell wall–degrading and other hydrolytic enzymes presumed to be deployed during infection to gain access to nutrition, but very few genes of this class have been directly connected to pathogenicity (Ma et al., 2013). Fungal pathogenic genes are commonly grouped into different categories such as formation of infection structures, cell wall degradation, toxin biosynthesis and signaling, and are responsible for fungal behaviours as spore attachment and germination, infection and colonization of the host, suppression or disruption of host defense mechanisms (Sutherland et al., 2012). A total of 1802 fungal DEGs, homologous to sequences assigned to Fol, was identified in both melon genotypes during FOM1.2 infection. Among these, 728 and 322 DEGs were found in the susceptible genotype CHT at 24 and 48 hpi respectively, while 143 and 609 DEGs were detected in the resistant genotype NAD at 24 and 48 hpi, respectively. The higher abundance of pathogen transcripts observed predominantly at the early stage of infection during the compatible interaction (1050 vs 752 DEGs in the compatible and incompatible interaction, respectively) indicated that the fungus was free to penetrate and colonize the xylem of CHT, while the prompt NAD response weakened fungal virulence, leading it to tolerate pathogen invasion. Several FOM1.2 DEGs that have previously been associated with pathogenicity in other fungi have been identified. Table 6 reports a list of selected fungal transcripts differentially expressed in melon. Many pathogenic fungi rely mainly on the production of CWDEs to enter plant tissue (Łazniewska et al., 2010). The CWDEs are involved in fungus penetration by means of hydrolysis of lignocellulosic components of the host cell wall and in the successively nutrient acquisition of plant wall polysaccharides (Aragona and Valente, 2013). The FOM1.2 DEGs identified *in planta* and representing putative virulence factors were prevalently assigned to groups as CWDEs, cytoskeleton components, mitochondrial proteins, vacuolar transport proteins, peroxisomal proteins and transcription factors. In Fol, genes that encode CWDEs have been identified as crucial factors for fungal colonization (Di Pietro et al., 2003; Sestili et al., 2011). CWDE comprise xylanases degrading hemicellulose, exopolygalacturonases and pectin methylesterases digesting pectin polymers, endoglucanases acting on cellulose and

polysaccharide deacetylases, which cleave acetyl substituents in polysaccharide components (Łazniewska et al., 2010). Twenty-seven *CWDE genes* with the highest expression values were identified in CHT at 24hpi (Figure 17), 14 of which could be considered as potential virulence factors since they resulted to be specific for either the plant and the time point. Among them deserve special consideration: *endoglucanase genes* (FOXG_02912, FOXG_04120, FOXG_05654, FOXG_07527, FOX_G09643, FOXG_13415) that are involved in fungus penetration and nutrient acquisition (Valente et al., 2011); *pectin* and *pectate lyase* precursor genes (FOXG_13249 and FOXG_16516) that degrade the peptic components of the plant cell wall; the *PG1 gene* (FOXG_14695) that encodes the major in vitro extracellular endopolygalacturonase of the tomato vascular wilt pathogen Fol (Benedetti et al., 2011) and the exopolygalacturonases *Pgx1* and *Pgx4 genes* (FOXG_08862 and FOXG_15415), that may have an important function in pathogen-plant interactions since they are generally not inhibited by plant PGIPs (García-Maceira et al., 2001). The identification of an *endo-1,4-beta-xylanase 2 precursor gene* (FOXG_09638), induced only in CHT, suggest that this gene is not peculiar to FOM race 1 as reported by Sestili et al. (2011) and that its induction is probably activated by the interaction with the host. The cytoskeleton-related proteins *fimbrin* (FOXG_05565), *actin* (FOXG_04579) and *α*- and *ß-tubulin* (FOXG_00655 and FOXG_06228) were predominantly identified in CHT at 24 hpi and in NAD at the later time point, confirming that the rearrangement of cytoskeleton is crucial for pathogen invasion (Upadhyay and Shaw, 2008). A *class V chitin synthase gene* (ChsVb) (FOXG_04163), pathogenicity determinant in *F. oxysporum* and mediator of protection against plant defense compounds (Madrid et al., 2003), was found at 24 and 48 hpi in CHT and NAD respectively, while, the *chitin synthase 4 gene* (FOXG_00113) was specific for the resistant plant at 48 hpi. A high number of FOM1.2 DEGs *in planta*, 72 and 43 DEGs in the compatible and incompatible interactions respectively, suggested a large involvement of the mitochondrial proteins in pathogenesis. On the contrary of that reported by Inoue et al. (2002) no significant differences were observed in the transcript amounts of the pathogenicity involved mitochondrial protein F*ow1* (FOXG_11292) during the time course of the experiment. Vacuolar proteins related genes, i.e. *vacuolar protein sorting-associated protein 21* (FOXG_09392), *vacuolar calcium ion transporter* (FOXG_03015) and *hypothetical protein similar to vacuolar H+/Ca2+ exchanger* (FOXG_4182), were predominately regulated in CHT at 24 hpi, suggesting a dynamic vacuolar trafficking during the infection. The vacuole is likely to play a variety of roles in supporting host colonization and infection by both mammalian and plant fungal pathogens (Palmer, 2011). The transcription factor *fow2* (FOXG_06378), a Zn(II)2Cys6-type transcription regulator, appeared to be unnecessary for vegetative growth, conidiation in cultures, and for carbon source utilization, but is essential for pathogenesis (Sutherland et al., 2012). It was expressed in CHT at 24 hpi and in NAD at 48 hpi, with a higher fold change in the susceptible plant (Table 6). Two fungal *PEX genes*, FOXG_09532 and FOXG_07868, expressed in CHT at 24 and 48 hpi respectively, could be putative pathogenicity factors being the peroxisomal function and fatty acid metabolism required for fungal virulence (Michielse et al., 2009). Of particular concern is the SCF (Skp1, Cullins, and F-box proteins) E3 ubiquitin ligase-mediated ubiquitin-proteasome system complex. Fungal SCF complexes have been reported to regulate a variety of cellular functions, including virulence (Wang et al., 2011). Duyvesteijn et al. (2008) asserted that Frp1 interacts with Skp1, suggesting the involvement of an SCF ubiquitin ligase complex in pathogenicity. The *fpr1* gene is required for virulence since it codes for a PR-1 like protein constitutively expressed during infection and under different culture conditions (Prados-Rosales et al., 2012). The RNA-Seq data obtained showed that the *frp1* gene (FOXG_00058) had similar expression values in the compatible and incompatible interactions only at 24 hpi and 48 hpi, respectively, while the *skp1* gene (FOXG_01898) was expressed only in CHT at 24 hpi. The lack of Frp1/Skp1 interaction in the resistant plant NAD could be responsible for the weakening of FOM1.2 virulence and

confirm the involvement of this complex in the pathogenicity. However, in agreement with the scarcity of available FOM genomic data, the majority of FOM1.2 DEGs (58.7%) were assigned to the conserved, predicted and hypothetical protein categories in both genotypes and time points considered. These not-annotated sequences are of great importance since they could allow the identification of new potential virulence factors. The BLAST analysis, carried out to find FOM1.2 DEGs homologous with the already known *Fusarium* pathogenicity genes (http://csb.tongji.edu.cn/efg/search/pathogenic.py), showed 22 transcripts (pvalue $<10^{-3}$) expressed in CHT at 24 hpi. Among them, 7 genes resulted to be unique: *NADH dehydrogenase iron-sulfur protein 2, mitochondrial precursor NOS1* (FOXG_00680), *casein kinase II subunit alpha* (FOXG_00997), *FK506-binding protein 1* (FOXG_08379), hypothetical *protein similar to HET-C2* (FOXG_04150), *hypothetical protein similar to maltose porter* (FOXG_13625), a *conserved hypothetical protein similar to TRI14* (FOXG_10792) and a *hypothetical protein similar to* MAPKKK (FOXG_09411), establishing their role as effector of FOM1.2 pathogenicity. The limited number of pathogenicity orthologous found was due to the lack of FOM1.2 genomic data. These results confirmed that the susceptible plant is early colonized by the pathogen that proliferates and exerts its virulent action, while NAD triggers a prompt defense response that weakened the virulence of FOM1.2 and lead it to tolerate the pathogen.

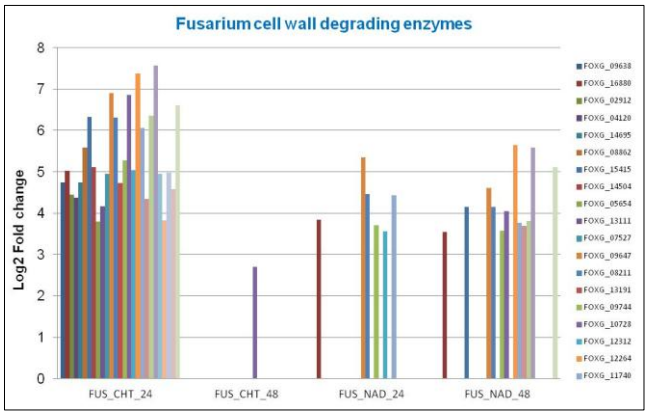


Figure 17. Different patterns of fungal CWDEs transcript accumulation between the two genotypes

Table 6. List of selected fungal transcripts differentially expressed in melon

FUNCTIONAL CATEGORY	ID *Fusarium oxysporum* database v F02.20	GENE NAME	ANNOTATION	log2 Fold Change			
				CHT 24 hpi	CHT 48 hpi	NAD 24 hpi	NAD 48 hpi
CDWEs	FOXG_09638		endo-1,4-beta-xylanase 2 precursor [Source:BROAD_F_oxysporum;Acc:FOXG_0963	4,75	-	-	-
	FOXG_16880		endoglucanase 3 precursor [Source:BROAD_F_oxysporum;Acc:FOXG_16880]	5,02	-	3,83	3,55
	FOXG_02912	EG-1	Endoglucanase type C [Source:UniProtKB/Swiss-Prot;Acc:P46237]	4,45	-	-	-
	FOXG_04120		endoglucanase-4 precursor [Source:BROAD_F_oxysporum;Acc:FOXG_04120]	4,38	-	-	-
	FOXG_14695	PG1	Endopolygalacturonase [Source:UniProtKB/TrEMBL;Acc:Q14TY6]	4,74	-	-	-
	FOXG_08862	PGX1	Exopolygalacturonase [Source:UniProtKB/TrEMBL;Acc:Q14TL1]	5,58	-	-	-
	FOXG_15415	PGX4	Exopolygalacturonase [Source:UniProtKB/TrEMBL;Acc:Q14TT9]	6,32	-	-	4,14
	FOXG_14504	XYL2	Family F xylanase [Source:UniProtKB/TrEMBL;Acc:O59938]	5,11	-	-	-
	FOXG_05654		hypothetical protein similar to endo-1,4-beta-glucanase [Source:BROAD_F_oxyspor	3,79	-	-	-
	FOXG_13111		hypothetical protein similar to endo-beta-1,4-mannanase [Source:BROAD_F_oxyspo	4,17	-	-	-
	FOXG_07527		hypothetical protein similar to endoglucanase [Source:BROAD_F_oxysporum;Acc:F	4,94	-	-	-
	FOXG_09647		hypothetical protein similar to endoglucanase [Source:BROAD_F_oxysporum;Acc:F	6,91	-	5,34	4,61
	FOXG_08211		hypothetical protein similar to endoglucanase B [Source:BROAD_F_oxysporum;Acc	6,30	-	4,46	4,15
	FOXG_13191		hypothetical protein similar to exopolygalacturonase [Source:BROAD_F_oxysporum	4,73	-	-	-
	FOXG_09744		hypothetical protein similar to pectate lyase [Source:BROAD_F_oxysporum;Acc:FO	5,27	-	3,71	3,57
	FOXG_10728		hypothetical protein similar to pectate lyase [Source:BROAD_F_oxysporum;Acc:FO	6,86	2,70	-	4,05
	FOXG_12312		hypothetical protein similar to pectate lyase [Source:BROAD_F_oxysporum;Acc:FO	5,03	-	3,56	-
	FOXG_12264		hypothetical protein similar to pectate lyase A [Source:BROAD_F_oxysporum;Acc:F	7,37	-	-	5,65
	FOXG_11740		hypothetical protein similar to pectate lyase C [Source:BROAD_F_oxysporum;Acc:F	6,06	-	4,44	3,77
	FOXG_13103		hypothetical protein similar to pectate lyase D [Source:BROAD_F_oxysporum;Acc:F	4,34	-	-	3,69
	FOXG_05948		pectate lyase B precursor [Source:BROAD_F_oxysporum;Acc:FOXG_05948]	6,36	-	-	3,81
	FOXG_11739		pectate lyase precursor [Source:BROAD_F_oxysporum;Acc:FOXG_11739]	7,57	-	-	5,58
	FOXG_13249		pectate lyase precursor [Source:BROAD_F_oxysporum;Acc:FOXG_13249]	4,95	-	-	-
	FOXG_16516		pectin lyase precursor [Source:BROAD_F_oxysporum;Acc:FOXG_16516]	3,83	-	-	-
	FOXG_09643		Putative endoglucanase type B [Source:UniProtKB/Swiss-Prot;Acc:P46236]	4,99	-	-	-
	FOXG_13415	XYL3	Putative endoglucanase type F [Source:UniProtKB/Swiss-Prot;Acc:P46239]	4,58	-	-	-
	FOXG_00480	CBH-C	Putative exoglucanase type C [Source:UniProtKB/Swiss-Prot;Acc:P46238]	6,60	-	-	5,11
CYTOSCHELETON	FOXG_02339		Beta-tubulin [Source:UniProtKB/TrEMBL;Acc:Q6GUE1]	4,40	-	-	-
	FOXG_05565		fimbrin [Source:BROAD_F_oxysporum;Acc:FOXG_05565]	3,79	-	-	-
	FOXG_04579		actin [Source:BROAD_F_oxysporum;Acc:FOXG_04579]	-1,68	-4,14	-	-2,11
	FOXG_06228	BENA	Beta-tubulin [Source:UniProtKB/TrEMBL;Acc:Q64F21]	2,63	-	-	3,61
	FOXG_00956		hypothetical protein similar to alpha-tubulin B [Source:BROAD_F_oxysporum;Acc:F	4,54	-	-	3,84
	FOXG_00655		tubulin alpha-2B chain [Source:BROAD_F_oxysporum;Acc:FOXG_00655]	4,88	-	-	3,81
	FOXG_01463		tubulin alpha-2B chain [Source:BROAD_F_oxysporum;Acc:FOXG_00655]		2,83	-	-
CHITIN SYNTHASE	FOXG_05290		chitin synthase 1 [Source:BROAD_F_oxysporum;Acc:FOXG_05290]	4,58	-	-	3,94
	FOXG_04163		ChsVb [Source:UniProtKB/TrEMBL;Acc:A7L5W4]	4,99	-	-	4,26
	FOXG_04162	CHSV	Class V chitin synthase [Source:UniProtKB/TrEMBL;Acc:Q873Z8]	4,63	-	-	4,98
	FOXG_00113		chitin synthase 4 [Source:BROAD_F_oxysporum;Acc:FOXG_00113]	-	-	-	3,62
MITOCONDRION	FOXG_11292	FOW1	Putative mitochondrial carrier protein [Source:UniProtKB/TrEMBL;Acc:Q8TGD1]	3,45	3,27	3,72	4,18
VACUOLE	FOXG_00773		vacuolar ATP synthase 98 kDa subunit [Source:BROAD_F_oxysporum;Acc:FOXG_	-	-	-	3,57
	FOXG_00954		vacuolar ATP synthase subunit B [Source:BROAD_F_oxysporum;Acc:FOXG_00954	3,95	-	-	-
	FOXG_01284		cerevisin precursor [Source:BROAD_F_oxysporum;Acc:FOXG_01284]	5,11	-	-	4,16
	FOXG_03015		vacuolar calcium ion transporter [Source:BROAD_F_oxysporum;Acc:FOXG_03015]	3,95	-	-	-
	FOXG_03408		vacuolar ATP synthase subunit E [Source:BROAD_F_oxysporum;Acc:FOXG_03408	3,80	-	-	-
	FOXG_04182		hypothetical protein similar to vacuolar H+/Ca2+ exchanger [Source:BROAD_F_oxys	3,83	-	-	3,94
	FOXG_09392		vacuolar protein sorting-associated protein 21 [Source:BROAD_F_oxysporum;Acc:F	3,69	2,72	-	-
	FOXG_12714		vacuolar protease A precursor [Source:BROAD_F_oxysporum;Acc:FOXG_12714]	4,26	-	-	-
	FOXG_09534	VTS1	protein VTS1 [Source:BROAD_F_oxysporum;Acc:FOXG_09534]	4,29	-	-	-
TF	FOXG_06378	FOW2	Zn(II)2Cys6 transcription factor [Source:UniProtKB/TrEMBL;Acc:Q0WXM3]	4,04	-	-	3,79
PEROXISOME	FOXG_07868		peroxiredoxin HYR1 [Source:BROAD_F_oxysporum;Acc:FOXG_07868]	-	3,28	-	-
	FOXG_05960		hypothetical protein similar to peroxisomal carrier protein [Source:BROAD_F_oxyspe	3,88	-	-	4,28
	FOXG_12260		peroxidase/catalase 2 [Source:BROAD_F_oxysporum;Acc:FOXG_12260]	4,71	3,17	3,56	4,04
	FOXG_17180		peroxidase/catalase 2 [Source:BROAD_F_oxysporum;Acc:FOXG_17180]	6,67	-	-	4,50
	FOXG_10111		peroxiredoxin 1 [Source:BROAD_F_oxysporum;Acc:FOXG_10111]	4,10	3,52	-	3,93
	FOXG_06347		peroxisomal hydratase-dehydrogenase-epimerase [Source:BROAD_F_oxysporum;A	6,19	-	3,98	-
	FOXG_09532		hypothetical protein similar to peroxisomal carrier protein [Source:BROAD_F_oxyspe	4,02	-	-	-
SCF COMPLEX	FOXG_01898	SKP1	SCF complex subunit Skp1 [Source:UniProtKB/TrEMBL;Acc:Q6B956]	3,70	-	-	-
	FOXG_00058	FRP1	Frp1 [Source:UniProtKB/TrEMBL;Acc:Q6B957]	4,79	-	-	4,36
	FOXG_09795	PR1	PR-1-like protein [Source:UniProtKB/TrEMBL;Acc:C8CJN6]	5,17	-	-	4,07
	FOXG_09446		hypothetical protein similar to F-box and WD repeat-containing protein [Source:BRO	3,88	-	-	3,69
	FOXG_05190		E3 ubiquitin-protein ligase pub1 [Source:BROAD_F_oxysporum;Acc:FOXG_05190]	4,47	-	-	4,60

- Not differentially expressed genes.

Note: The numbers indicate log2fold change of differentially expressed genes.

4.10 Time course analysis and validation of selected DEGs

RNA-Seq data highlight an earlier activation of the immune response of the resistant plant NAD against FOM1.2. Based on similar patterns of transcript accumulation during the experimental time course, genes associated with resistance were selected. Differential transcript levels both for downstream effector genes and genes involved in regulatory functions in the susceptible genotype CHT ranged from abolishment of gene transcription at 24 hpi to lower gene expression at 48 hpi (Figure 18).

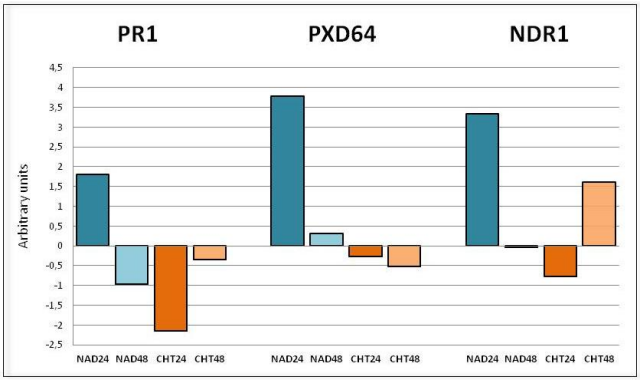


Figure 18. Different patterns of transcript accumulation during the experimental time course between the two genotypes.

The expression trends of 10 melon sequences mainly involved in defense and stress responses and 3 fungal transcripts differently expressed *in planta* were confirmed by quantitative real-time PCR (qRT-PCR). In NAD inoculated plants, the gene expression was higher than the correspondent mock-inoculated samples. Among the selected melon genes, three NA sequences (MELO3C002948, MELO3C010155 and MELO3C015129), expressed only in the resistant line, were characterized by a similar trend of transcript accumulation, being activated at 24 hpi (log_2-fc from 4.44 to 12.70) and repressed at 48 hpi. (Figure 19). The analysis of the *PR thaumatin-like protein* (MELO3C003144), the *E3 ubiquitin ligase ATL6-like* (MELO3C007732), the *pectinesterase* (MELO3C005291), two *LRR receptors* (MELO3C017541 and MELO3C023962) *genes* and the *PR1 gene* (MELO3C017497) showed a higher expression value at 24 hpi in NAD than in the susceptible plant CHT (Figure 20). The qRT-PCR expression profiles of *anchyrin gene* (MELO3C000909) differed from the correspondent RNA-Seq profiles showing a significant transcript accumulation in CHT at 48 hpi (Figure 21). The different expression levels between RNA-Seq and qRT-PCR could be caused either by the bioinformatics process performed in the RNA-Seq analysis (Łabaj et al., 2011) and by the dynamic nature of the transcriptome (Martínez-Gómez et al., 2012). Some effector genes (e.g. FOW2, FRP1 and SKP1) required for FOM1.2 virulence in melon have also been validated. Although with different log_2-fold change values (log_2-fc from 2.00 to 6.12), they resulted highly expressed in CHT along the whole experimental timeline while in NAD they were slightly activated only at 48 hpi (Figure 22). As expected, these fungal transcripts were never recovered in the mock-inoculated samples. The time course qRT-PCR analysis allowed us to validate the expression changes of particular interesting genes. Although the expression values were, in most cases, lower in RNA-Seq than in qRT-PCR experiments, the data were largely concordant, confirming the reliability of the results and a substantial agreement in the fungal-induced variations for transcripts accumulation in the selected genes.

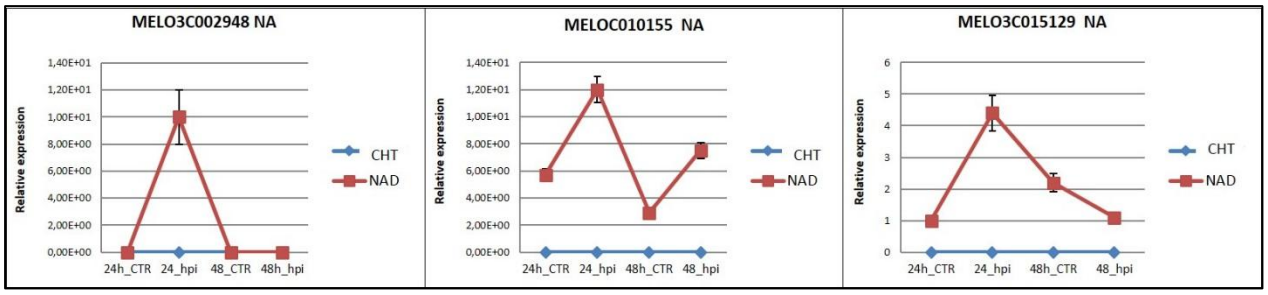


Figure 19. NAD-specific unknown DEGs for highlighting new putative candidate resistance genes.

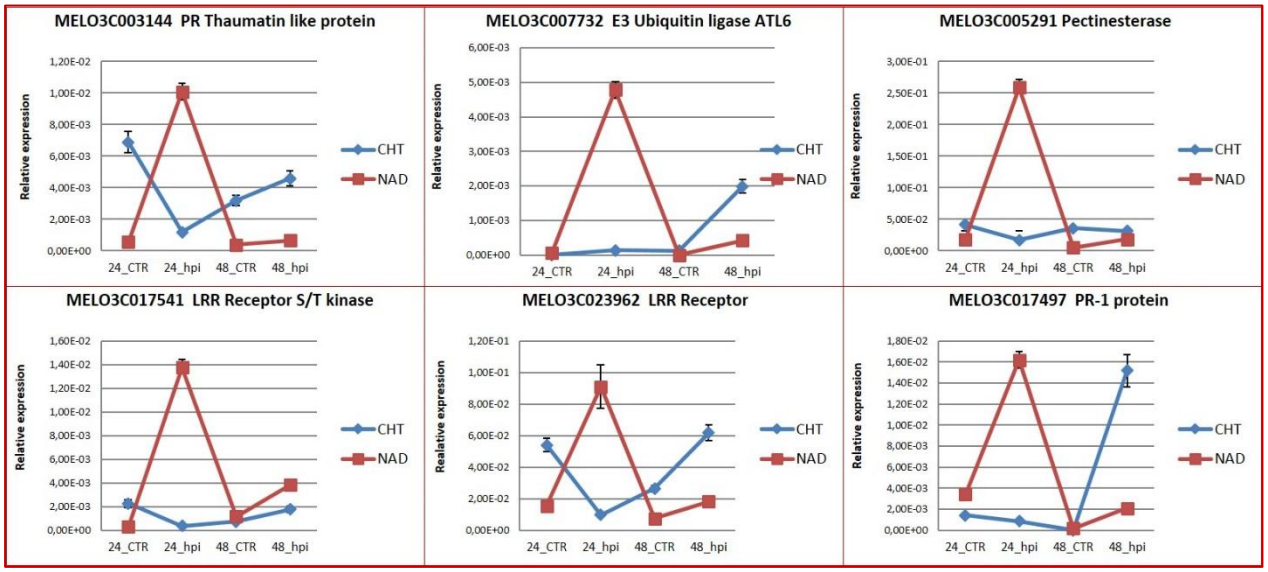


Figure 20. Expression patterns of genes involved in the resistance in susceptible and resistant melon genotypes after FOM1.2 inoculation as determined by qRT-PCR.

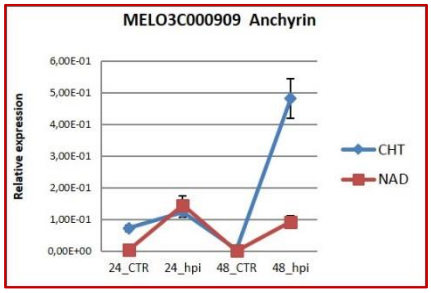


Figure 21. The qRT-PCR expression profiles of anchyrin gene were not validated since it differed from the correspondent RNA-Seq profile showing a significant transcript accumulation in the susceptible plant.

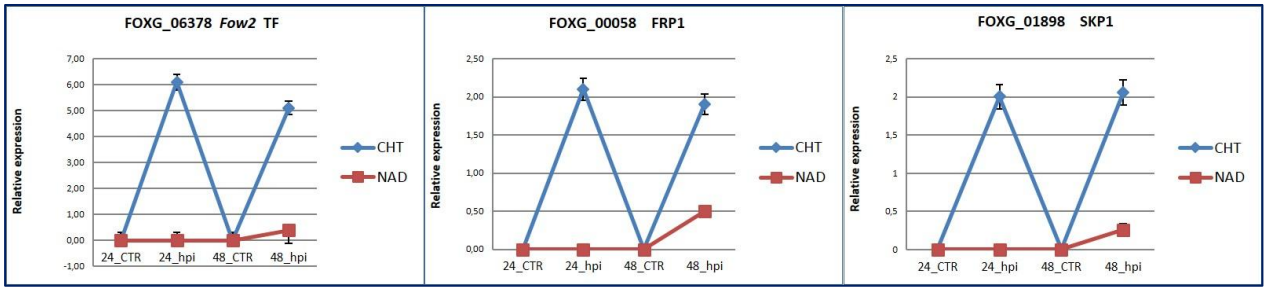


Figure 22. Fusarium DEGs expressed in melon could represent FOM1.2 virulence factors.

4.11 Conclusions

In this study, the whole transcriptomes of compatible and incompatible melon-FOM1.2 interactions were characterized by RNA-Seq analysis at 24 and 48 hpi. The differences in the obtained DEGs between the resistant DH line NAD and the susceptible cultivar CHT were more evident at the early stage of infection, confirming the prompt activation of defense responses in NAD. The time-course RNA-seq analysis results showed that:

1) NAD-specific response was characterized by a fine regulation of a battery of processes including Ca^{2+} mediated up-regulation of PTI/ETI, cell wall reorganization and crosstalk among hormone signaling pathways.
2) Different types of RLK and putative defense-related genes were specifically induced at different time points, suggesting a distinct role of these genes at specific stage(s) following FOM1.2 infection.
3) Several of the selected DEGs were induced during the incompatible interaction at 24 hpi to ensure specific resistance as well as in the compatible interaction at 48 hpi to centrally contribute to basal resistance, suggesting that timing, more than abundance, differentiate resistance and susceptibility.
4) NAD defense against FOM1.2 was mainly signalled by JA and ET, with the ET-mediated signaling pathway activated earlier than the JA mediated networks. Like in *Arabidopsis* and tobacco ET signaling was probably activated by MAPKK4/5 in melon.
5) JA/ET and auxin could positively interact in NAD resistance to FOM1.2.
6) Exogenous auxin improved the melon resistance to FOM1.2 and could provide a potential new treat for controlling Fusarium wilt in melon. Since exogenous auxin could be absorbed by melon, it is difficult to determine the relationship between endogenous and exogenous auxin, and thus, additional studies are needed to fully understand these observations.
7) During the infection process, FOM1.2 induced water stress by occluding the xylem vessels. The resistant plant was able to overcome this problem overexpressing at 24 hpi genes implicated in the ABA signaling pathway.
8) Resistance effectors of NAD quickly weaken fungal virulence, but not impaired its vegetative growth and conidiation. The FOM1.2-specific DEGs in *planta* could probably act as fungal virulence effectors.

The extensive transcriptome analysis performed will contribute to a better understanding of the complex defense system in melon towards FOM1.2, providing candidate resistance genes whose biological function could be further in depth investigated.

IN VITRO PLANT REGENERATION AND TRANSFORMATION

4.12 Medium optimization for shoot regeneration

Regeneration of a patterned multi-cellular organism from the adult somatic tissue is a well-known phenomenon. Compared with animals, plants have a profound capacity to regenerate organs from their differentiated somatic tissues through the manipulation of plant hormones (Su et al., 2011). Previous studies showed that *in vitro* manipulation of melon is difficult; genotype is the main essential factor influencing the efficiency of plant regeneration (Ficcadenti and Rotino, 1995; Choi et al., 2012). Furthermore, plant growth regulator (PGR) types, concentrations and combinations have significant effects on plant regeneration (Kumar et al., 2011). Auxin and cytokinin play important roles during plant growth and in particular in some developmental processes, such as cell specification during embryogenesis and formation and maintenance of meristems that are essential to establish the whole plant body (Möller and Weijers 2009; Su et al., 2011). Based on the finding that a high ratio of cytokinin/auxin promotes bud neoformation (Skoog and Miller 1956), in all used media the level of cytokinin was 100 times higher than the auxin. Cotyledon explants were cultured on three media containing different concentrations of cytokinin (BAP) and auxin (IAA). The plant growth regulator concentrations used were BAP 1.0 - IAA $1.0 \cdot 10^{-2}$ mg/L, BAP 1.2 - IAA $1.2 \cdot 10^{-2}$ mg/L and BAP 1.3 - IAA $1.3 \cdot 10^{-2}$ mg/L in the medium A, B, and C respectively. Moon et al. (2000) indicated the 0.5 mg/L BA and 0.1 mg/L IAA concentrations as optimal, but a more recent study reported the achievement of the highest rate of shoot induction by using 1.5 mg/L BA and 0.1 mg/L IAA hormone level (Zhang et al., 2011). Keng and Hoong (2006) indicated that auxin was not necessary for multiple shoot induction from the nodal segments of honeydew melon, and the presence of auxin induced the formation of friable calli. These discrepancies on the ratio of hormone used and the rate of shoot induction is probably due to the different melon genotypes used. From the obtained results, it is obvious that the chosen combinations of cytokinin (BAP) and auxin (IAA) were appropriate for melon shoot regeneration. Swelling of explants was observed within 2 weeks (Figure 23a) and after 20 days shoot formations emerged on the cut edges and/or in the central region of the cultured slices (Figure 23b, c). On the contrary to what reported by Choi et al. (2012), the explants kept in darkness for 15 days became yellowing and vitreous suggesting that this treatment is not useful for shoot induction from melon cotyledons. The light culture gave the best percentage of forming calluses and explant necrosis appeared reduced. After 30 days of culture the percentage of explants with at least one shoot and the number of shoot per explants were recorded (Figure 23e, f, g). In Table 7 is reported a ranking of the genotypes based on their performance on the different regeneration media used. The bud regeneration frequency varied among the genotypes and medium composition (Figure 24a, b), confirming that the genetic background plays a central role in melon shoot formation. In the medium A and B, the genotype CHT developed the highest number of shoot/explants (4.1 and 4.4 in A and B respectively), while for the medium C the cultivar Vedrantais gave the best result (2.4), even if with a lesser number of shoots than the one obtained by CHT on the other medium/hormone combinations. Among the cultivars, CHT resulted to be the best in terms of developed shoots for medium A and B, while in medium C it ranked after Vedrantais and NAD (Figure 24a). Among the DH lines, NAD was the most responsive and produced the highest number of shoot/explants in all the three media (2.9, 1.9 and 1.6 for the medium A, B and C respectively) (Figure 24a). The DH-L6 had ever the lowest efficiency rate (0.4, 0.3 and 0.1 shoots/explant for the medium A, B and C respectively), resulting to be in this case the most recalcitrant melon genotype among those tested (Figure 24a). With the exception of the DH-L2 (1.1 shoots/explants), the medium C showed the lowest potential in shoot induction, suggesting that, while maintaining the same

ratio, even small changes (increase or decrease) in cytokinin and auxin concentrations produce significantly effect on shoots formation (Figure 24a). This data is consistent with that reported by Moon et al. (2000) for the oriental melon cultivar "Tongilwand". The cultivar Isabelle exhibited not significant dissimilar shoot formation ability (0.5, 0.5 and 0.8 shoots/explant in the medium A, B and C respectively) and plant regeneration rate (24.0%, 30.3% and 22.5% regenerated plants in the medium A, B and C respectively), irrespective of the medium of culture used (Figures a, b). Notwithstanding the cultivars developed the greater number of shoot/explants, the highest plant regeneration rate was achieved by all DH lines on the three medium considered (Figure 24b). The frequency of plant regeneration fluctuated from 24.0 to 84.5% depending on both melon genotypes and medium tested (Figure 24b). In particular, the DH-L6 regenerated 85.4% and 50.0% of plants on medium A and C respectively, and ranked after NAD (65.6%) on medium B (41.7%) (Figure 24b). Although the cultivar Vedrantais was able to develop the highest number of shoots on media C, it gave the best plant regeneration rate on medium A (Figure 24b). Despite the DH-L6 exhibited the lowest number of shoots on medium A, it showed the highest rate of plant regeneration (84.5%) (Figure 24b), suggesting a higher regeneration ability of this genotype. This result indicate that there is a negative correlation between the amount of the induced shoots and the number of regenerants obtained. The medium that induces more buds is not necessarily the most suitable to use since the regenerative potential of the developed shoots could be influenced by both genetic and environmental conditions. For stem elongation, several shoot buds were placed on a MS medium containing 0.5 mg/L BAP, but the appearance of vitreous elongated shoots was observed. In oriental melon, this step was indispensable for obtaining elongated shoots from small buds (Choi et al., 2012), on the contrary the genotypes under study completely lost their regenerative potential. Therefore this phase was avoided and, after 45 days of culture, 2-3 cm high shoots were excised from callus and directly rooted on hormone-free MS medium (Figure 23g, h, i). Over 90% of shoots formed roots after 2 weeks of culture, regenerating complete melon plantlets with well-developed shoot and root systems.

Figure 23. Stages of *in vitro* melon shoot development. a, b, c) Sketch buds and *primordia*. d, e, f) Shoots development and proliferation. g, h) Elongated shoot. i) Rooted shoots regenerating a complete melon plantlet.

Table 7. Ranking of the genotypes based on their performance on the regeneration media

medium A		**medium B**		**medium C**	
shoots/explant	**plant regeneration**	**shoots/explant**	**plant regeneration**	**shoots/explant**	**plant regeneration**
CHT	DH-L6	CHT	NAD	Vedrantais	DH-L6
NAD	Vedrantais	Vedrantais	DH-L6	NAD	DH-L2
Vedrantais	NAD	NAD	CHT	CHT	NAD
DH-L2	DH-L2	DH-L2	Isabelle	DH-L2	Isabelle
Isabelle	Isabelle	Isabelle	Vedrantais	Isabelle	Vedrantais
DH-L6	CHT	DH-L6	DH-L2	DH-L6	CHT

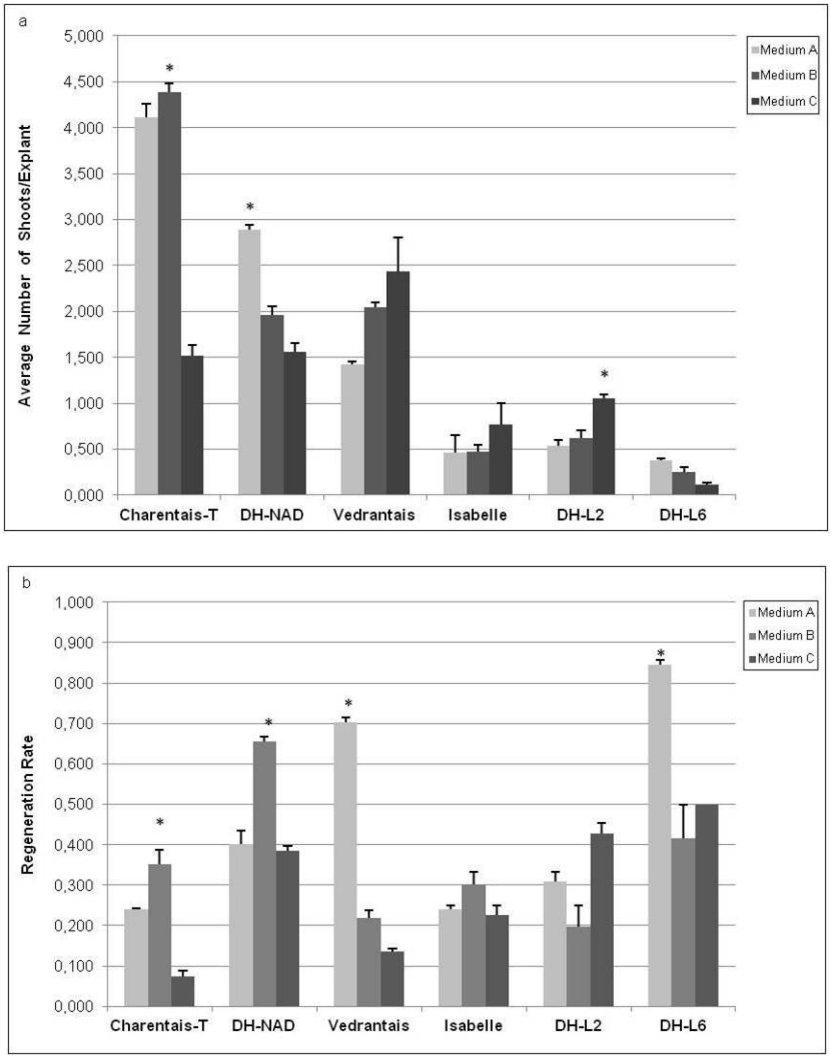


Figure 24. The histograms highlighted the differences in the development of shoots/explants (a) and in the rate of complete melon plant regeneration (b) among the three *media* for each genotype. *Duncan's multiple range test, significant means at p = 0.05

4.13 Assessment of clonal fidelity of the regenerated plants by RAPD analysis

Somaclonal variation has been the greatest threat to the widespread application of tissue culture techniques to agricultural crops (Akin-Idowu et al., 2009). In the early days of tissue culture application, it was assumed that the plants produced would be clonal, that means genetic uniformity. Currently, it is well known that 'off-types' are often produced and the heritable mutations passed on to subsequent sexual reproduction cycles (Sahijram et al., 2003). Although somaclonal variation is a useful source of genetic variability for crop improvement, it reduces the commercial value of plants and is undesirable in plant regeneration system and genetic transformation. Therefore is indispensable to ascertain the genetic stability of the regenerated plants for a better and potential use of tissue-culture techniques. Molecular markers represent a more reliable tool than phenotypic observations for evaluating genetic changes. Numerous studies on somaclonal variation analysis have been developed using PCR-based techniques such as RAPD, SSR and AFLP (Lakshmanan et al., 2007; Cuesta et al., 2010; Mehta et al., 2011; Nadha et al., 2011; Pandey et al., 2012). RAPD is one of the most used for its simplicity and cost-effectiveness. It requires no prior knowledge of the genome that is being analyzed and it allows the amplification of discrete fragment that can be used as genetic markers to determine differences among individuals at the DNA level (Jayanthi and Mandal, 2001). The RAPD technique has been extensively used to assess genetic variability generated by *in vitro* procedures (Rani et al., 1995; Roy et al., 2012; Devi et al., 2013). Although the successful assessment of RAPD profiles generated requires validation through repeated experiments or a crosschecked by using another marker system, it can be used for quick evaluation of clonal variability within the regenerated plants, by random scanning of the whole genome. In order to confirm the genetic stability of each melon genotype, the genomic DNA of 9 regenerated plants (three per each medium randomly chosen) was compared to the DNA of the corresponding mother plants. Twenty RAPD primers were screened based on the DNA amplification patterns of the six parental genotypes. All of them produced amplification products. The primer OP A-01 gave the highest number of bands (15 bands) while the primer OP E-09 produced the lowest (5 bands). The quantification of polymorphic loci is an important parameter used in the genetic fidelity analysis of a population. All the 20 primers produced monomorphic bands showing a high degree of genetic stability within the *in vitro* regenerated and propagated melon plants. No polymorphic bands were observed among all the regenerants and the respective control plants, revealing the genetic homogeneity and true-to-type nature of the *in vitro*-raised clones. Figures 25a and b showed representative NAD amplification patterns obtained with primers OP A-01 and OP A-04, respectively. Callus formation is a genetic mosaic of cells (Edallo et al., 1981), the ones able to regenerate plants should be more resistant to somaclonal variation during the *in vitro* callus induction and maintenance processes (Vilaça de Vasconcelos 2008). Addition of growth regulators to culture medium is known to have influence on the frequency of the karyotype alterations in cell cultures. Plant tissues grown *in vitro* are vulnerable to certain degree of genetic variations under the presence of potent plant growth regulators like thidiazuron (Bhattacharyya et al., 2014). In particular, the use of different combination of BA, ABA and IAA increased the ploidy level of *C. melo* var. *inodorus* (Ren et al., 2013). Recently, it was reported that lower level of auxin greatly reduced variability in regenerated *Hordeum* plants, as it compared with a high concentration (Jikku, 2013). The present study shows that a high ratio of BAP/IAA (low auxin level) prevents the development of somaclonal variability within the *in vitro* melon regenerants obtained from cotyledonary explants. Although the ploidy level could have been deeper investigated with other methods (flow cytometry and tissue staining), the use of molecular markers as RAPD ascertained the genetic stability of the regenerated plants, because the presence of polymorphism could be explained as different copy number of the corresponding

DNA loci in the samples under study (Stegnii et al., 2000). The results confirmed the discriminating power of this technique to determine the somaclonal variation level in melon.

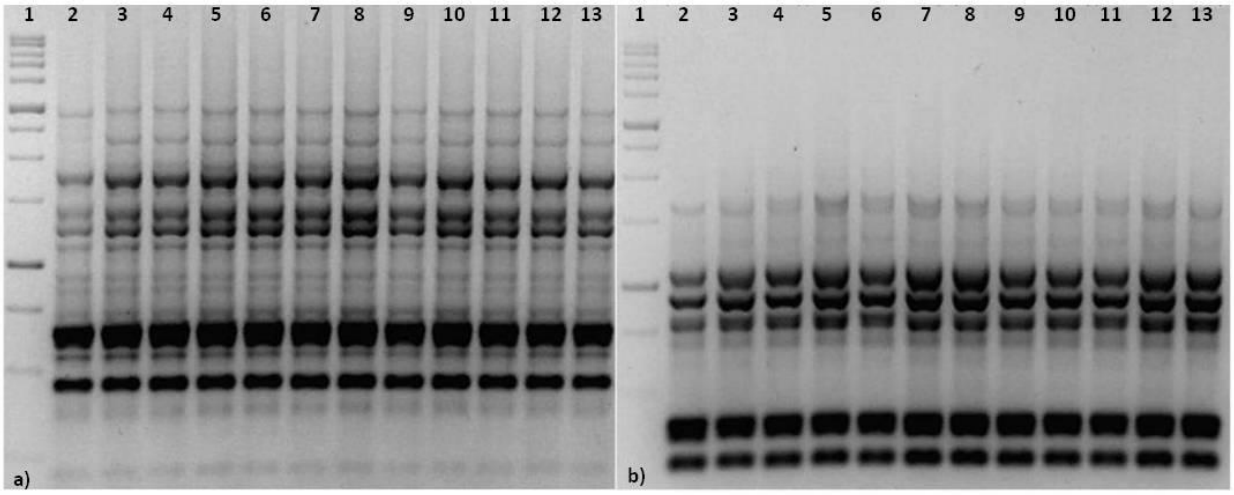


Figure 25. RAPD analysis of mother and regenerated plants of the melon DH line NAD. Lane 1: 1Kb marker; lanes 2-4: mother plants; lanes 5-7: regenerants cultured on medium A; lanes 8-10: regenerants cultured on medium B; lanes 11-13: regenerants cultured on medium C. a) Amplification products obtained with OP A-01; b) Amplification products obtained with OP A-04.

4.14 Ploidy determination

In melon, explant type was reported to be a major factor impacting the efficiency of regeneration, but at the same time affect the ploidy level of the regenerants (Ren et al., 2013). Guis et al. (2000) obtained more than 80% of tetraploid regenerated plants from cotyledon explants of *C. melo* L. var. *cantalupensis* (cultivar. Vedrantais). Other explant types such as somatic embryos, adventitious shoots, shoot primordia, and hypocotyls have resulted in lower frequencies of polyploid plants compared to cotyledonary explants (Ezura et al., 1992, 1994; Guis et al., 2000; Curuk et al., 2003; Nuňez-Palenius et al., 2006; 2008). This is due to the propensity of cotyledonary tissue to undergo endopolyploidization that results from chromosomal endoreduplication without cell division (De storme et al., 2013). Hypocotyl tissue is an excellent source to maintain parental ploidy in regenerated plants but is more difficult to obtain regenerated plants (Ren et al., 2013). The spontaneous generation of tetraploid plants from diploid genotypes was found to occur universally in melon tissue culture and can be considered as a factor limiting the further development of the genetic manipulation of this species. This phenomenon is not restricted to melon and has been observed among other *Cucurbitaceae* species such as cucumber (Colijn-Hooymans et al., 1994). Melon plant ploidy level can be determined by cytological methods, such as counting the chromosome number using root squash tips. Feulgen Staining remains the gold standard for precise DNA image cytometry (Biesterfeld et al., 2011). The ploidy level (2n=24) among wild type and 9 random *in vitro* maintained regenerants (three derived from each medium) was confirmed by Feulgen staining (chromosome counting in root tip). Melon chromosomes are smaller in comparison with other plants, which complicated the easy chromosome observation, but plant nuclei had, as far as could be determined, the normal chromosome number of 2n =24 (Figure 26).

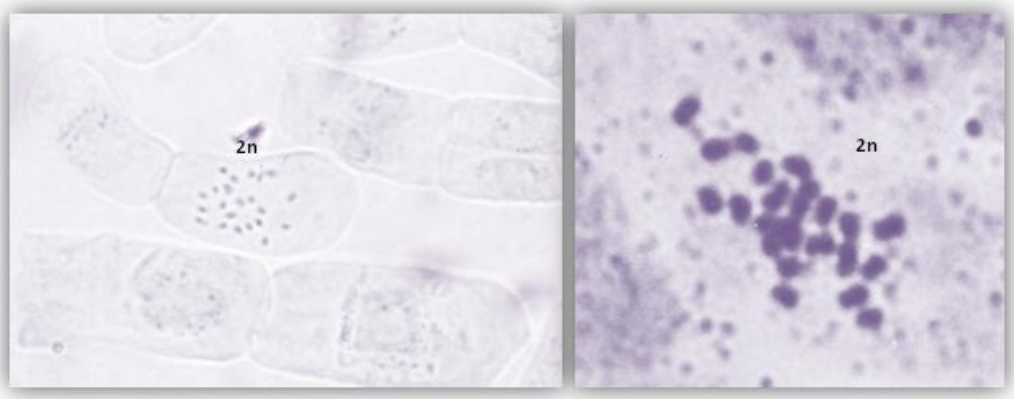


Figure 26. Chromosomes of diploid cells from root tip of regenerated NAD plantlets.

4.15 Genetic transformation via *Agrobacterium tumefaciens*

After the successfully regeneration step, two avirulent *A. tumefaciens* strains, pC58 GV2260 and LB4404 GV3101, harboring the binary plasmid *pGUS-INT*, containing the *ß-glucoronidase* (GUS) and the *nptII* genes, were used for the transformation of NAD and CHT genotypes. *NptII*, one of the most commonly used selection marker gene for the screening of transgenic plants, encodes a phosphotransferase capable of phosphorylating aminoglycoside antibiotics, including kanamicin (km), geneticin (Gt), neomycin and paromomycin. To date, Km has been the most widely used of these aminoglycoside antibiotics in melon transformation protocols (Galperin et al., 2003; Nora et al., 2012). In plant transformation systems, however, the balance between antibiotic concentration to achieve stringent selection and genuine transgenic shoot regeneration is highly species and cultivar-dependent, thereby necessitating optimization of the chosen antibiotic(s) prior to transformation. The optimal concentrations of Km (75 and 100 mg/L) and Gt (5, 10, 30 mg/L) to use for melon shoot induction were first determined by adding them to solid LB medium for testing the bacterial growth. *Agrobacterium* growth was completely inhibited at 30 mg/L Gt. Therefore the transformed explants were cultured on medium B supplemented with 75 and 100 mg/L Km and 5 and 10 mg/L Gt. Petri et al. (2008) reported that Gt inhibited the regeneration of apricot leaves at almost all concentrations tested. Gt is a more active antibiotic and has been reported to be much more sensitive than Km in the regeneration and selection of *nptII*-transformed apple tissue (Norelli and Aldwinckle, 1993). However, following transformation melon cotyledon explants did not develop shoots. Within 2 weeks, explants became bleached or formed vitreous callus. The unique result, i. e. adventitious shoots formation, was obtained for the genotype CHT infected with *pGV3101* and cultured on medium B containing 10 mg/L Gt and 500 mg/L cefotaxime (Figure 27). Shoots appeared from the cut ends of the cotyledon explants within 2 weeks of culture initiation (Figure 27) but after other 2 weeks they became vitreous and bleaching, thus no melon plants were completely regenerated. The frequency of shoot regeneration was very low for CHT while no shoot formation was observed for the DH line NAD. The transformation events occurred in CHT explants were confirmed by the histochemical GUS activity assay. The explants sampled after 9 days of culture on selection medium resulted blue spotted (Figure 28). Numerous studies have shown that transformation efficiency is restricted by the choice of target genotype, suggesting that plant genetic factors determine responses to *Agrobacterium*-mediated transformation (Cogan et al., 2001). Plant genes that regulate the transformation process have been identified in a DH mapping population of *Brassica oleracea* (Cogan et al., 2002). Although genetic transformations of melon have been successful obtained for other cultivars (Fang and Grumet, 1993; Gui et al., 2000; Taler et al., 2004), so far haploid and DH plants have never been used in melon genetic transformation. The failure in transforming the DH line NAD highlights a strong evidence for a genetic basis of the susceptibility to *Agrobacterium*-mediated transformation. These results, although need to be deeper investigated, represent an important starting point for the rapid production of homozygous transgenic DH line either for the expansion of genetic variation in breeding material or for functional genomics studies in melon.

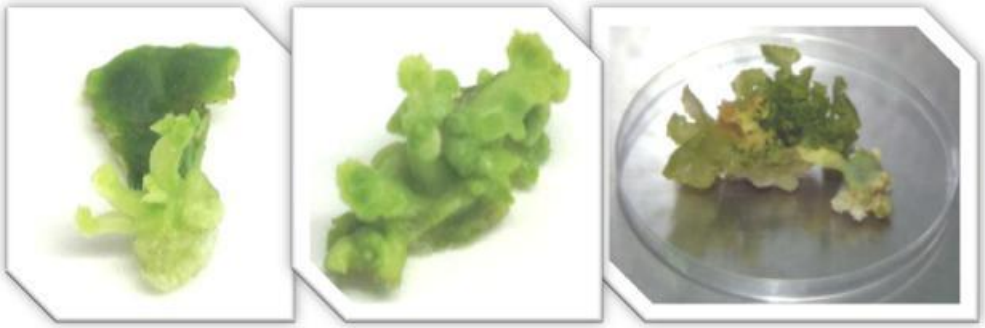


Figure 27. CHT cotyledonary explants transformed with avirulent *A. tumefaciens* harboring the binary plasmid pGV3101 produced adventitious shoots on regeneration medium B containing 10 mg/L Gt and 500 mg/L Cefotaxime.

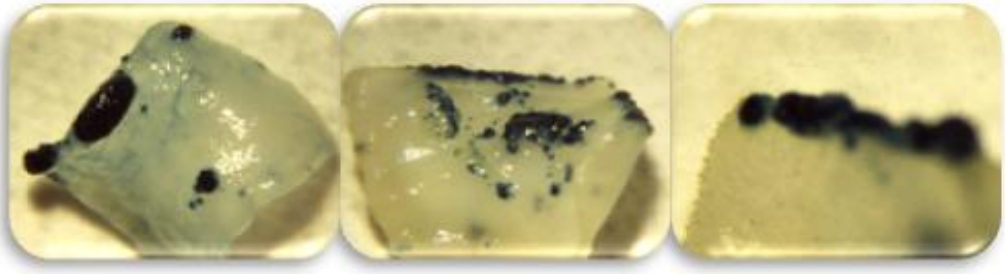


Figure 28. GUS activity was detected histochemically as blue-stained tissue in CHT cotyledonary explants.

4.16 Conclusions

An efficient, simple and reproducible *in vitro* plant regeneration protocol for *C. melo* var *cantalupensis* cultivars and DH lines has been developed. The results confirmed that plant growth regulators play an important role in plant morphogenesis and the process of bud neoformation requires a high hormonal balance between cytokinin and auxin (low auxin level). The DH-lines showed a good plant regeneration rate, however comparable with that of the cultivars. The present study provides the first report on the genetic stability of micropropagated melon plants using RAPD analysis. Since there was no change in the RAPD banding patterns of regenerants analyzed in comparison with the control plants, it can be concluded that the protocol standardized here could be successfully used for large-scale *in vitro* clonal propagation and conservation of melon and also applicable for improving the efficiency of genetic transformation of these varieties.

5. MATERIALS AND METHODS

TRANSCRIPTOME ANALYSIS

5.1 Plant material and *in vivo* inoculation procedure

The resistant DH line NAD (Ficcadenti et al., 1995) and the "differential host" CHT, that lacks any resistance genes to FOM, were used. Seeds were surface sterilized with 1% NaOCl for 20 min and incubated in sterile distilled water at 4°C overnight. The seeds were pre-germinated on filter paper and seedlings were cultivated in plastic pots filled with sterilized soil in the greenhouse at 25 ± 2°C with 80-90% relative humidity. The FOM1.2 strain ISPaVe1018 was obtained from the fungal collection of the Plant Pathology Research Center (CRA-PAV, Rome). The inoculum preparation and the followed artificial inoculation procedure were reported in Sestili et al. (2011) (Figure 29). For both genotypes, a total of 50 plants was used to investigate the fungal vascular colonization and 10 plants per each time point were pooled and used for RNA extraction and transcriptome analysis. All plantlets were phenotypically screened at 8, 15, 21 and 35 dpi.

Figure 29. 1, 2) At the four-to-five true leaf stage plants were removed from the pots. 3, 4) Washing of the roots. 5, 6) Pruning of the roots. 7) Conidial suspension of FOM1.2. 8, 9) Inoculation: the roots were dipped in the fungal suspension. 10, 11) The inoculated seedlings were re-planted into new pots. 12) The inoculum was poured directly on the ground.

5.2 RNA extraction procedure and library preparation

Total RNA was isolated from infected and mock-treated plants at 24 and 48 hpi using a combination of TRIzol reagent (Invitrogen) and a Qiagen RNeasy® Minikit (Qiagen, Valencia, CA, USA; http://www.qiagen.com), according to the manufacturer's instructions. Two biological replicates for each genotype and treatment were used. Stems of a pool of five plants per each biological replicates (~2 g) were excised with a sterile razor blade, dehydrated in liquid nitrogen and stored at -80°C. For library preparation 4 µg of total RNA were used following the manufacturer's instructions of the Illumina TruSeq RNA sample preparation kit (FC-122-1001). The amplified and purified libraries were analyzed on the 2% low range ultra-agarose gel (BIO-RAD) for selecting an average size of 300 bp. RNA quality (RIN > 8) and library size were assayed on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA; http://www.agilent.com). The accurate concentration of the libraries was assessed by Real-Time PCR (Applied Biosystem).

5.3 Illumina GAIIx Sequencing

Libraries were sequenced on a Genome AnalyzerIIx (CRA-Genomics Research Center, Fiorenzuola d'Arda, PC) to produce 51 bp single-end reads. Samples were run multiplexing two libraries per lane. The individual libraries were pooled using the same amount of each library. FastQ file generation was performed by CASAVA v1.8.2.

5.4 Quantitative RT-PCR analysis

QRT-PCR of 13 genes selected DEGs was assessed. First strand cDNA was synthesized from 100 ng of the samples used in the RNA-Seq experiment, by using the "High Capacity cDNA Reverse Transcription Kit" (Applied Biosystem). Primers were designed using the Primer3 Software (http://frodo.wi.mit.edu/primer3/) (Table 8) and the specificity was checked by blasting the sequences in the NCBI database. Gene abbreviations were assigned based on the corresponding *A. thaliana* orthologous abbreviation on the TAIR (the *Arabidopsis* information resource) website (http://www.arabidopsis.org) or were self-assigned when no *A. thaliana* orthologous was found. The melon constitutively expressed *ribosomal protein L2 gene* was used as reference gene (Sestili et al., 2014). All qRT-PCR reactions were carried out on a Rotor-Gene 6000 machine (Qiagen), with the following thermal cycling profile: 50°C for 2 min and 95°C for 2 min, followed by 40 cycles at 95°C for 3 s and 60°C for 30 s. Melting curve analysis was performed to verify single product formation with temperature ranging from 55°C to 95°C by increasing of 1°C every step. All reactions were performed in a total volume of 10 µl which contained 30 ng cDNA sample, 5 µl 1× SYBR® Select Master Mix (Applied Biosystem) and 0.2 µl (20 µM) of each primer. For each sample, two biological replicates were analyzed in independent runs and a no-template control was included for each gene. Intra-assay variation was evaluated by performing all amplification reactions in triplicate. The quantification cycle (Cq) was automatically determined using Rotor-Gene 6000 Series Software, version 1.7 as reported in Sestili et al. (2014).

Table 8. Primer sequences of genes for qRT-PCR expression profile validation

ID	ANNOTATION	Fw_LEFT PRIMER	Rv_RIGHT PRIMER	AMPLICON LENGHT (bp)	PRODUCT Tm°C
MELO3C002948	NA	CCAGTTCGGGTCAGGGAAAA	GCCTCGGGTTTAACAGTGGA	91	84.8
MELO3C010155	NA	CGACGATGAACACCACACAG	ATCTTCCGTCTGTCGTGCAG	81	81.8
MELO3C015129	NA	TCTGGCAAACGCTCTCCAAT	TTTTGCGAGCGTTTGGTGAG	142	84.4
MELO3C000909	Anchyrin	GGGTTAACAGCTCTTGACATCC	GGCGTCTGTGAGAGTCTCTG	81	80.3
MELO3C023962	LRR Receptor	CGTTTGACCACCTTGAAGCG	TCTAACCCGATCCGGTTCCT	93	82.7
MELO3C017541	LRR Receptor-like serine/threonine protein kinase	TAATGGGTTTTCCGGGGAGC	TCGGAACCACACCTTCGAAG	92	82.0
MELO3C003144	PR Thaumatin like protein	ACACCAAAAGACGGGGGTTT	AGTTGCAGCCTTGTCTACCC	103	82.9
MELO3C017497	PR	AGCAGCTGCAACTACCTCTG	CACACCAGGCGTTGTTTGAG	92	81.5
MELO3C007732	E3 Ubiquitin protein ligase ATL6 like	TGGAATGTGCCGTTTGCTTG	GTCGATGCAAGGGGGATGAA	95	82
MELO3C005291	Pectinesterase	CGCAGCGTTAACGGATCAAG	GCTTCTTCACCTTCTCCGCT	104	83.6
FOXG_06378	transcription factor fow2	CCAAGCTCTCCGTATCGCAT	CCCATCTGAGGCATGGTTGT	109	82.1
FOXG_00058	frp1	CTGTTGAGACGCCGCAAAAA	ATTTGGAGGCAAGTCGGAGG	129	83.5
FOXG_01898	skp1	TTGATGTCGGCTGCAAGACT	CCTCGGGAGTGAAGTCGTTC	99	82.7

5.5 Exogenous auxin supply

To investigate the effects of auxin on disease development, both NAD and CHT plants were treated with exogenous IAA applications. Ten seedlings for each genotype were treated with the hormone two days before the infection (pre-treatment) and then 24 and 48 hours after FOM1.2 inoculation. As controls, five plants were inoculated with FOM1.2, but without treatment with auxin, and five plants were mock-inoculated. Each plant was single potted with the same amount of peat mixture (1.5 kg). The IAA solution was directly injected into the soil by means of a syringe plunged exactly below the crown to thoroughly flood the roots. Two different IAA concentrations were used: 5 and 10 mM. For the artificially inoculation procedure the method reported in Sestili et al. (2011) was followed. Plants were daily phenotypically screened to assess the effects of auxin on morphology and plant fitness. The development of disease-related symptoms was also registered.

5.6 Bioinformatics methods

5.6.1 Mapping of Illumina reads

Raw fastQ files were checked for contaminants and low quality bases and contaminants were trimmed out with Cutadapt software (Martin, 2011). Contaminant-free, filtered reads were mapped with Bowtie/Tophat. The spliced read mapper Tophat version 1.4.1 (Trapnell et al., 2012) was used to map reads to *Cucumis melo* L. genome (CM version 3.5) (Garcia-Mas et al., 2012). Minimum and maximum intron lengths of 40 and 50000 were used, respectively. Read counts were collected with HTSeq version 0.5.3 (http://www-huber.embl.de/users/anders/HTSeq) in "union" mode.

5.6.2 DEGs calling

DESeq version 1.10.1 (Anders and Huber, 2010) and DESeq2 version 1.2.8 Bioconductor packages were used to call melon and FOM1.2 DEGs, respectively. Both DESeq and DESeq2 implement models based on negative binomial distribution, which was developed with special attention to cope with biological variance. For Deseq, the cutoff for considering a gene expressed was set to 0.5 RPKM. DESeq parameters for dispersion estimation were: method "pooled" and sharing Mode "fitOnly". DESeq2 parameters were set to fit type="parametric", BetaPrior=T and independent filtering was allowed. The FDR threshold for DEG calling was set to 0.05 for both DESEq and DESeq2.

5.6.3 Melon sample clustering

DESeq-normalized melon samples were transformed with function VST (DESEq package) and heatmaps were created with heatmap.2 function as available in the "gplots" Bioconductor.

5.6.4 GO enrichment analyses

Gene ontology terms for melon genes were obtained by running BLAST2GO (Götz et al., 2008) using as query melon proteins (CM3.5.MELO.3C) against NR database, with the following annotation parameters: E-value hit filter $1.E^{-10}$, Annotation cut-off 55, Go weight 5, Hsp-Hit coverage cutoff 20. Gene reference sets were genes above expression thresholds (RPKM > 0.5).

5.6.5 MapMan tool

MapMan figures were obtained by first running the mercator tool (http://mapman.gabipd.org/web/guest/ mercator) with default parameters to assign MapMan bins to melon transcripts. Log2 fold changes as obtained from DESeq output were used as MapMan input to represent expression changes.

5.6.6 KEGG Map

The Bioconductor package pathview version 1.6.0 (Luo and Brouwer, 2013) was used to generate relevant KEGG pathway pictures incorporating color-coded expression values. Pathview parameters were set as default ones and the limit parameter was set as: limit=list (gene=5, cpd=1).

5.7 Study of melon colonization

5.7.1 FOM1.2-GFP inoculum preparation

A FOM1.2 isolate expressing the *gfp* reporter gene, collected in Israel (Zvirin et al., 2010), was maintained on potato dextrose agar (PDA; Difco Laboratories) plates supplemented with hygromycin B (100 µg m/L) at 25°C. A conidial suspension, started from an agar-plate culture, was grown in a flask with 250 mL liquid medium (0.5% PDA and 0.5% yeast extract, Difco Laboratories) at 25 °C on a rotary shaker at 180 rpm for 5 days. The concentration of conidia in the suspension culture was adjusted to 10^6 conidia/ml for plant inoculation with sterile distilled water. Ten seedlings of both NAD and CHT genotypes were artificially inoculated following the procedure reported in Sestili et al. (2011).

5.7.2 FOM1.2-GFP microscopic examination

Observations were performed at 24 and 48 hpi on infected and control plants. GFP signals were analysed with a fluorescence microscope using a mercury-vapor lamp with an excitation filter of 510 nm and equipped with a Nikon digital camera. Images were taken using Plan Apox10, 40 and 100x water immersion lens. From each seedling, several hand sections were prepared from the middle of the main and secondary roots and the crown, and then inspected under the microscope. As reported in Zvirin et al. (2010), a seedling was scored as colonized at a given tissue section when fluorescent mycelium (regardless of the amount) was recorded in the section examined. All the procedure was performed at University of Camerino (MC), under the supervision of Prof. Patrizia Ballarini.

IN VITRO PLANT REGENERATION AND TRANSFORMATION

5.8 Plant materials

Based on their interaction with FOM1.2, three cultivars of *C. melo* var. *cantalupensis* (CHT, Vedrantais and Isabelle) and three DH melon lines (NAD, DH-L2 and DH-L6), parthenogenetically originated by using irradiated pollen (Ficcadenti et al., 1995), were employed to evaluate their morphogenetic response to different hormone concentrations (Table 9). An average of 30 seeds per genotypes was cultured. The de-coated seeds were dipped in 70% (v/v) alcohol for 30–60s, surface sterilized in 3% aqueous (v/v) commercial bleach (1.5% sodium hypochlorite) for 30 min and rinsed (three or four times) with sterile distilled water. The seeds were placed on Petri dishes containing Murashige and Skoog (MS) (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose and 7 g/L agar (Duchefa Biochemie, Haarlem, the Netherlands). The same basal medium was used for plant regeneration.

Table 9. Selected melon genotypes and their interaction with FOM1.2

Melon genotypes	Bot. var. *cantalupensis*	Response to FOM1.2
Charentais-T	cultivar	Susceptible
Vedrantais	cultivar	Susceptible
L2	doubled-haploid line	Susceptible
NAD	doubled-haploid line	Resistant
L6	doubled-haploid line	Resistant
Isabelle	cultivar	Resistant

5.9 Plant regeneration

The 6-days old cotyledons were detached, cut along the borders and across the midrib to obtain 2 segments of about 5mm^2. The explants were then cultured for shoot induction on a Petri dish containing MS supplemented with BAP and IAA in different concentrations. Each of six genotypes was cultured on 3 induction media, henceforth marked with A, B, and C. The MS "A" contained BAP 1.0 and IAA $1.0 \cdot 10^{-2}$ mg/L, the MS "B" contained BAP 1.2 and IAA $1.2 \cdot 10^{-2}$ mg/L and the MS "C" contained BAP 1.3 and IAA $1.3 \cdot 10^{-2}$ mg/L. All media were sterilized by autoclaving at 121°C (1 atm) for 15 min after adjusting the pH to 5.8. The filter-sterilized (0.2 µm, Whatman) growth regulators were added to the autoclaved substrates. The cultures were incubated at 25±2°C under a 16-h photoperiod (50 μmol/m^2 s^1) and the subcultures to a fresh medium started after 2 weeks of cultures. A dark treatment for 15 days was also supplied to test the influence on shoot formation. The numbers of developing shoots, buds or multiple shoot *primordia* were scored after 30 days of culturing. The visible elongated shoots (2-3 cm) were removed from the slice after approximately 45 days and then transferred onto the growth

regulator-free MS basal medium for rooting. Furthermore for each genotype, 10 shoot buds were placed on a MS medium containing only cytokinin (BAP 0.5 mg/L) to induce stem elongation (Choi et al., 2012). The obtained regenerated plantlets were grown *in vitro* at the same condition of culturing.

5.10 Experimental design and statistical analysis

A total of 120 explants originated from 30 seeds for genotype was used. The procedure was repeated two times along then course of experiment (~ 6 weeks). For each genotype, six explants per Petri dish were cultured and a total of 30 explants was placed on each medium and maintained under a 16h photoperiod. Furthermore, 30 explants were cultured in the dark for 15 days. Both the numbers of shoots/explants and the plant regeneration rate were scored. The data were subjected to Two-way analysis of variance (ANOVA) and the differences among means were compared by Duncan's new multiple range test (= 0.05) using the STATISTICA (StatSoft, Tulsa, OK, USA) version 7.0 Software.

5.11 DNA isolation

Genomic DNA of young leaves was isolated from three random chosen plants obtained from each regeneration medium that in turn it means a total of 9 plants per genotype. As control, 3 donor mother plants for each genotype were also used. The PureLink® Genomic Plant DNA Purification kit (Invitrogen) was used according to the manufacturers' instructions. DNA concentration was assessed at 260 nm using the NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Thermo Scientific). Total DNA quality was determined using the A260/A280 and A260/A230 absorption ratios (NanoDrop, Technical support bulletin T009). The DNA integrity was checked by electrophoresis on 0.8% agarose gel.

5.12 RAPD analysis

The genetic stability of the regenerated plants was assessed by PCR-based RAPD analysis. Twenty decamer RAPD primers were selected according to the number and consistency of amplified fragments (Table 10). All reactions were performed in a total volume of 20 µl which contained 30 ng genomic DNA, 4 µl 5X Buffer, 1.6 µl $MgCl_2$, 0.2 µl Taq and 0.3 µM of each primer. Amplification was carried out in a DNA Thermal Cycler GeneAmp® PCR System 9700 (Applied Biosystem) with the following cycling profile: 1 cycle of 3 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. The last cycle was followed by 5 min extension at 72°C. PCR products were separated by electrophoresis in 1.2% agarose gel. The size of the amplicons was estimated using 1 kb DNA ladder (Euroclone) and photographed by the Kodak 1D 3.6 documentation system. Two independent amplification reactions were performed with the 20 primers for all the 6 genotypes. RAPD fragments were scored for presence (1) or absence (0) of homologous fragments over all accessions. Only clear and reproducible bands were considered. The polymorphic bands were intended as absent in some sample in frequency greater than 1%; change in band intensity was not considered as polymorphism.

Table 10. Sequences of RAPD primers

Sr. No.	Primer	Sequence (5' - 3')
1	OP A-01	CAGGCCCTTC
2	OP A-04	AATCGGGCTG
3	OP A-05	AGGGGTCTTG
4	OP A-09	GGGTAACGCC
5	OP A-12	TCGGCGATAG
6	OP A-13	CAGCACCCAC
7	OP A-20	GTTGCGATCC
8	OP B-07	GGTGACGCAG
9	OP B-15	GGAGGGTGTT
10	OP C-14	TGCGTGCTTG
11	OP C-17	TTCCCCCCAG
12	OP C-18	TGAGTGGGTG
13	OP E-01	CCCAAGGTCC
14	OP E-09	CTTCACCCGA
15	OP G-04	AGCGTGTCTG
16	OP G-06	GTGCCTAACC
17	OP G-13	CTCTCCGCCA
18	OP G-15	ACTGGGACTC
19	OP I-03	CAGAAGCCCA
20	OP L-09	TGCGAGAGTC

5.13 Analysis of the DNA-content of nuclei during mitosis in melon root tips (Feulgen staining)

The Feulgen staining for DNA Ploidy Analysis is a histochemical technique for quantifying the nuclear DNA content in cells (chromosome counts). Feulgen's reaction is based on the specific reaction between Schiff's reagent and the 2-deoxyribose nucleic acids. The optimal degree of hydrolysis of DNA is reached only after the purine and pyrimidine bases have been cleaved off, resulting in the release of a maximum number of aldehyde groups. The reproducibility of Feulgen's reaction is of essential importance for the reliability of the DNA measurement. Root tips were collected from different randomly selected regenerants and control plants at different stages of *in vitro* culture. When roots were 4-5 mm long (Figure 30), a section was taken off and placed 2 hours in (1:2) bromonaphthalene:distilled water and then in Carnoy's fixative overnight at room temperature. Hydrolysis was carried out at 60°C in 1N HCl for 13 min, and then stained with Schiff's reagent (Carlo Erba®) for one hour in the dark. Root tips were squashed in a drop of 45% acetic acid and mounted on a single slide. Microscopic examinations for chromosome counting were performed using a Leica_DMLB optic microscope, with a 100x magnification; the displayed images were photographed by Minolta X-300S camera. It is enough to find a single diploid nucleus for declaring the diploidy state of the plant.

Figure 30. Melon roots were 4-5 mm long and appeared opaque-white and yellowish pointed.

5.14 Melon genetic transformation via *Agrobacterium tumefaciens*

Two disarmed strains of *Agrobacterium tumefaciens*, C58 GV2260 and LB4404 GV310, harboring the binary plasmid *pGUS-INT* and containing the *ß-glucoronidase* (GUS) and the *nptII* genes, were used for the genetic transformation of melon cotyledonary explants. A glycerol stock of *A. tumefaciens* was thawed and then streaked onto a Luria Broth (LB) solid medium containing 150 mg/L Km and 150 mg/L rifampicin. A single isolated colony was used to initiate a culture in 200 mL LB liquid medium. Cotyledon explants of NAD and CHT genotypes obtained from 6-day-old seedlings (30 seeds per two repetitions) were immersed in 30 ml suspension of *Agrobacterium* (OD_{600} 0.6–0.7) and acetosyringone 20 mM and then incubated for 30 minutes at 25°C with shaking (100 rpm). The explants were then blotted on Whatman n81 paper for 10 min, placed abaxially on the culture medium and co-cultured on MS for 2 days in the dark. After co-cultivation, the explants were then transferred on MS supplemented with BAP 1.2 and IAA $1.2 \cdot 10^{-2}$ mg/L (medium B) containing 500 mg/L Cefotaxime and the selection agent for 2 weeks without subculture to fresh medium. Different concentrations of Km (75, 100 mg/L) and Gt (5, 10, 30 mg/L) were added on the *Agrobacterium* culture medium for comparing their efficiency as selection agents and to evaluate the maximum concentration that not inhibits the bacterial growth. The histochemical GUS assay on the transformed explants was performed according to Jefferson et al. (1987).

6. REFERENCES

Acharya K, Awadhesh KP, Arvind G, Sanjay K, Anil KS, Paramvir SA (2013). Overexpression of *Camellia sinensis* Thaumatin-Like Protein, CsTLP in Potato Confers Enhanced Resistance to *Macrophomina phaseolina* and *Phytophthora infestans* Infection. *Molecular Biotechnology*, 54(2):609-622.

Adhikari TB, Wallwork H, Goodwin SB (2004). Microsatellite Markers Linked to the Stb2 and Stb3 Genes for Resistance to *Septoria tritici* Blotch in Wheat. *Crop Sci*, 44:1403-1411.

Aggarwal D, Kumar A, Reddy MS (2010). Shoot organogenesis in elite clones of *Eucalyptus tereticornis*. *Plant Cell Tiss Organ Cult*, 102:45–52.

Akasaka-Kennedy Y, Tomita K, Ezura H (2004). Efficient plant regeneration and Agrobacterium-mediated transformation via somatic embryogenesis in melon (Cucumis melo L.). *Plant Sci*, 166:763–769.

Akin-Idowu PE, Ibitoye DO and Ademoyegun OT (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology*, 8(16):3782-3788.

Almagro L, Gómez Ros LV, Belchi-Navarro S,. Bru R, Ros Barceló A,. Pedreño MA (2009). Class III peroxidases in plant defence reactions. *Journal of Experimental Botany*, 60(2):377–390.

An SH, Sohn KH, Choi HW, Hwang IS, Lee SC, Hwang BK (2008). Pepper pectin methylesterase inhibitor protein CaPMEI1 is required for antifungal activity, basal disease resistance and abiotic stress tolerance. *Planta*, 228(1):61–78.

Anders S and Huber W (2010). Differential expression analysis for sequence count data. *Genome biology*, 11: R106.

Anders S, Pyl PT, Huber W, Anders S, Huber W (2014). HTSeq – A Python framework to work with high-throughput sequencing data HTSeq – A Python framework to work with high-throughput sequencing data. *Bioinformatics*, doi http//dx.doi.org/101101/002824:0–5.

Antolìn-Llovera M, Ried MK, Binder A, et al. (2012). Receptor kinase signaling pathways in plant-microbe interactions. *Annu Rev Phytopathol*, 50:451–73.

Aragona M and Valente MT (2013). Endoglucanase expression and virulence in plant fungal pathogens. In The Fungal Cell Wall. Edited by Mora-Montes HM. New York: Nova Publishers; 2013:253–274.

Bagnaresi P, Biselli C, Orru` L, Urso S, Crispino L, et al. (2012). Comparative transcriptome profiling of the early response to *Magnaporthe oryzae* in durable resistant vs susceptible rice (Oryza sativa L.) genotypes. *PLoS ONE*, 7(12): e51609.

Bari R and Jones JDG (2009). Role of plant hormones in plant defense responses. *Plant Mol Biol*, 69:473–488.

Bhattacharyya P, Kumaria S, Diengdoh R, Tandon P (2014). Genetic stability and phytochemical analysis of the in vitro regenerated plants of *Dendrobium nobile Lindl.*, an endangered medicinal orchid. *Meta Gene*, 2:489–504.

Beckers GJ and Spoel SH (2006). Fine-Tuning Plant Defence Signalling: Salicylate versus Jasmonate. *Plant Biol (Stuttg),* 8(1):1-10.

Bednarek P, Pislewska-Bednarek M, Svatos A, Schneider B, Doubsky J, Mansurova M, Humphry M, Consonni C, Panstruga R, Sanchez-Vallet A, Molina A, Schulze-Lefert PA (2009). Glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science, 323(5910):101-106.

Belisario A, Luongo L, Corazza L, Gordon TR (2000). Indagini su popolazioni di *Fusarium oxysporum* f. sp. *melonis* in Italia. *Colture Protette*, 3:87-89.

Benedetti M, Leggio C, Federici L, De Lorenzo G, Pavel NV, Cervone F (2011). Structural Resolution of the Complex between a fungal Polygalacturonase and a Plant Polygalacturonase-Inhibiting Protein by Small Angle X Ray Scattering. *Plant Physiol,* 157(2):599-607.

Bent AF (1996). Plant disease resistance genes: function meets structure. *Plant Cell*, 8:1757-1771.

Berrocal-Lobo M and Molina A (2007). Arabidopsis defense response against *Fusarium oxysporum*. *Trends Plant Sci,* 13:145–50.

Biesterfeld S, Beckers S, Del Carmen Villa Cadenas M, Schramm M (2011). Feulgen staining remains the gold standard for precise DNA image cytometry. *Anticancer Res,* 31(1):53-8.

Blade JA, Franciso R, Queroz A, Regaloda PA, Ricardo CP, Veloso MM (2006). Immunolocalization of a class III chitinase in two muskmelon cultivars reacting differently to *Fusarium oxysporum* f.s.p. *melonis. J Plant Physiol*, 163:19–25.

Blanca JM, Cañizares J, Ziarsolo P, Esteras C, Mir G, Nuez F, Garcia-Mas J, Picó MB (2011). Melon transcriptome characterization: Simple Sequence Repeats and Single Nucleotide Polymorphisms discovery for High Throughput genotyping across the species. *The Plant Genome*, 4:118–131.

Bouwmeester K, de Sain M, Weide R, Gouget A, Klamer S, Canut H,et al.(2011). The lectin receptor kinase LecRK-I.9 is a novel *Phytophthora* resistance component and a potential host target for a RXLR effector. *PLoS Pathog*, 7:e1001327.

Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G (2010). A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *PNAS*, 107: 9452–9457.

Buchanan et al. (2003) Book: Biochemistry & Molecular Biology of Plants.

Castelblanque L, Marfa V, Claveria E, Martinez I, Perez-Grau L, Dolcet-Sanjuan R (2008). Improving the genetic transformation efficiency of *Cucumis melo* subsp. melo "Piel de Sapo" via *Agrobacterium*. In: Pitrat M (ed) Cucurbitaceae 2008. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of *Cucurbitaceae*, INRA, Avignon (France), May 21–24th, pp 627–631.

Chen JB, Zhang FR, Huang DF, Zhang LD, Zhang YD (2014). Transcriptome Analysis of Transcription Factors in Two Melon (*Cucumis melo* L.) Cultivars under Salt Stress. *Plant Physiology Journal,* 50 (2): 150-158.

Cheruvathur MK, Abraham J, Thomas TD (2013). Plant regeneration through callus organogenesis and true-to-type conformity of plants by RAPD analysis in *Desmodium gangeticum* (Linn.) DC. *Appl Biochem Biotechnol,* 169:1799–1810.

Chikh-Rouhou H, Gonzàlez-Torres R, Oumouloud A, Alvarez JM (2011). Inheritance of race 1.2 Fusarium wilt resistance in four melon cultivars. *Euphytica,* doi 10.1007/s10681-011-0411-4.

Choi JY, Shin JS, Chung YS, Hyung N (2012). An efficient selection and regeneration protocol for *Agrobacterium*-mediated transformation of oriental melon (Cucumis melo L. var. makuwa). *Plant Cell Tiss Organ Cult,* 110:133–140.

Chovelon V, Restier V, Giovinazzo N, Dogimont C, Aarrouf J (2011). Histological study of organogenesis in *Cucumis melo* L. after genetic transformation: why is it difficult to obtain transgenic plants? *Plant Cell Rep,* 30:2001–2011.

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM (2009). Glucosinolate metabolites requiredfor an Arabidopsis innate immune response. *Science,* 323, 95–101.

Clepet C, Joobeur T, Zheng Y, Jublot D, Huang M, Truniger V, Boualem A, Hernandez-Gonzalez ME, Dolcet-Sanjuan R, Portnoy V, Mascarell-Creus A, Caño-Delgado AI, Katzir N, Bendahmane A, Giovannoni JJ, Aranda MA, Garcia-Mas J, Fei Z (2011). Analysis of expressed sequence tags generated from full length enriched cDNA libraries of melon. *BMC Genomics,* 12:252.

Cogan I, Lynn R, King J, Kearsey J, Newbury J, Puddephat (2002). Identification of genetic factors controlling the efficiency of *Agrobacterium rhizogenes*-mediated transformation in *Brassica oleracea* by QTL analysis. *J. Theor Appl Genet,* 105(4):568-576.

Cogan N, Harvey E, Robinson H, Lynn J, Pink D, Newbury HJ, Puddephat I (2001). The effects of anther culture and plant genetic background on *Agrobacterium rhizogenes*-mediated transformation of commercial cultivars and derived double haploid *Brassica oleracea*. *Plant Cell Rep,* 20:755–762.

Colijn-Hooymans CM, Hakkert JC, Jansen J, Custers JBM (1994). Competence for regeneration of cucumber cotyledons is restricted to specific developmental stages. *Plant Cell Tiss. Org. Cult,* 39:211–217.

Corbacho J, Romojaro F, Pech JC, Latchè A, Gomez-Jimenez MC (2013). Transcriptomic events involved in melon mature-fruit abscission comprise the sequential induction of cell wall degrading genes coupled to a stimulation of endo and exocytosis. *PLoS ONE,* 8(3):e58363.

Cuesta C, Ordas RJ, Rodriguez A, Fernandez B (2010). PCR-based molecular markers for assessment of somaclonal variation in *Pinus pinea* clones micropropagated *in vitro*. *Biol Pl,* 54:435–442.

Curuk S, Elman C, Schlarman E, Sagee O, Shomer I, Cetiner S, Gray DJ, Gaba V (2002). A novel pathway for rapid shoot regeneration from the proximal zone of the hypocotyl of melon (Cucumis melo L.). *In vitro Cellular Developmental Biology-Plant,* 38:260-267.

Dahmani-Mardas F, Troadec C, Boualem A, Lévêque S, Alsadon AA, Aldoss AA, Dogimont C, Bendahmane A (2010). Engineering melon plants with improved fruit shelf life using the TILLING approach. *PLoS One*, 5(12):e15776.

Dai N, Cohen S, Portnoy V, Tzuri G, Harel-Beja R, Pompan-Lotan M, Carmi N, Zhang G, Diber A, Pollock S, Karchi H, Yeselson Y, Petreikov M, Shen S, Sahar U, Hovav R, Lewinsohn E, Tadmor Y, Granot D, Ophir R, Sherman A, Fei Z, Giovannoni J, Burger Y, Katzir N, Schaffer AA (2011). Metabolism of soluble sugars in developing melon fruit: a global transcriptional view of the metabolic transition to sucrose accumulation. *Plant Mol Biol,* 76:1–18.

Dangl JL and Jones JDG (2001). Plant pathogens and integrated defence responses to infection. *Nature*, 411, 826–833.

Dao TTH, Linthorst HJM, Verpoorte R (2011). Chalcone synthase and its functions in plant resistance. *Phytochem Rev,* 10:397–412.

De Cremer K, Mathys J, Vos C, Froenicke L, Michelmore R, Cammue BPA, De Coninck B (2013). RNAseq-based transcriptome analysis of *Lactuca sativa* infected by the fungal necrotroph *Botrytis cinerea*. *Plant, Cell and Environment,* 36:1992–2007.

Deleu W, Esteras C, Roig C, González-To M, Fernández-Silva I, Gonzalez-Ibeas D, Blanca J, Aranda MA, Arús P, Nuez F, Monforte AJ, Picó MB, Garcia-Mas J (2009). A set of EST-SNPs for map saturation and cultivar identification in melon. *BMC Plant Biol*, 9:90.

De Lorenzo G, Cervone F, Bellincampi D, Caprai C, Clark AJ, Desiderio A, Devoto A, Forrest R, Leckie F, Nuss L, Salvi G (1999). Polygalacturonase, PGIP and oligogalacturonides in cell-cell communication. *Biochemical Society Transactions*, 22:396-399.

De Storme N and Geelen D (2013). Sexual polyploidization in plants – cytological mechanisms and molecular regulation. *New Phytol,* 198(3):670–684.

Devi KD, Punyarani K, Singh NS, Devi HS (2013). An efficient protocol for total DNA extraction from the members of order Zingiberales - suitable for diverse PCR based downstream applications. *Springer Plus*, 2:669.

Di C, Zhang M, Xu S, Cheng T, An L (2006). Role of Polygalacturonase-inhibiting protein in plant defense. *Curr. Rev Microbiol.,* 32:91-100.

Di Pietro A, Madrid MP, Caracuel Z, Delgado-Jarana J, Roncero MIG (2003). *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Molecular Plant Pathology,* 4:315-325.

Diaz A, Fergany M, Formisano G, Ziarsolo P, Blanca J, Fei Z, StaubJE, Zalapa JE, Cuevas HE, Dace G, Oliver M, Boissot N, Dogimont C, Pitrat M, Hofstede R, van Koert P, Harel-Beja R, Tzuri G, Portnoy V, Cohen S, Schaffer A, Katzir N, Xu T, ZhangH, Fukino N, Matsumoto S, Garcia-Mas J, Monforte AJ (2011). A consensus linkage map for molecular markers and Quantitative Trait Loci associated with economically important traits in melon (Cucumis melo L.). *BMC Plant Biol,* 11:111.

Dirks R and van Buggenum M (1989). *In vitro* plant regeneration from leaf and cotyledon explants of *Cucumis melo* L. *Plant Cell Rep,* 7: 626-627.

Dodds PN and Rathjen JP (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet.*, 11(8):539-48.

Dong XNL, Wang M, Shen Q, Guo S (2012). Fusaric acid is a crucial factor in the disturbance of leaf water imbalance in *Fusarium* - infected banana plants. *Plant Physiology and Biochemistry,* 60:171-179.

Dong H and Cohen Y (2001). Extracts of killed *Penicillium chrysogenum* induce resistance against Fusarium wilt of melon. *Phytoparasitica*, 29:1-9.

Dowd C, Wilson LW, McFadden H (2004). Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with *Fusarium oxysporum* f. sp *vasinfectum*. *Mol Plant Microbe*, 17:654–667.

Duyvesteijn RG, van Wijk R, Boer Y, Rep M, Cornelissen BJ, Haring MA (2008). Frp1 is a *Fusarium oxysporum* F-box protein required for pathogenicity on tomato. *Mol. Microbiol,* 57:1051-1063.

Edallo S, Zucchinali C, Perenzin M, Salimini F (1981). Chromosomal variation and frequency of spontaneous mutation associated with *in vitro* culture and plant regeneration in maize. *Maydica,* 26: 39-56.

Eduardo I, Arús P, Monforte AJ (2005). Development of a genomic library of near isogenic lines (NILs) in melon (Cucumis melo L.) from the exotic accession PI161375. *Theor Appl Genet*, 112(1):139-48.

Egan AN, Schlueter J, Spooner DM (2012). Applications of next-generation sequencing in plant biology. *American Journal of Botany,* 99(2):175–185.

Encode project http://encodeproject.org/ENCODE/protocols/dataStandards/ENCODE_RNAseq_Standards_V1.0.pdf

Esteras C, Formisano G, Roig C, Diaz A, Blanca J, Garcia-Mas J, Gómez-Guillamón ML, López-Sese´ AI, Làzaro A, Monforte AJ, Pico´ B (2013). SNP genotyping in melons: genetic variation, population structure, and linkage disequilibrium. *Theor Appl Genet,* 126:1285–1303.

Ezura H and Fukino N (2009). Research tools for functional genomics in melon (Cucumis melo L.): current status and prospects. *Plant Biotech,* 26:359–368.

Ezura H, Amagai H, Kikuta I, Kubota M, Oosawa K (1995). Selection of Somaclonal Variants with Low-Temperature Germinability in Melon (Cucumis melo L). *Plant Cell Rep,* 14:684-688.

Ezura H and Oosawa K (1994). Ploidy of somatic embryos and the ability to regenerate plantlets in melon (Cucumis melo L). *Plant Cell Rep,* 14:107-111.

Ezura H, Amagai H, Yoshioka K, Oosawa K (1992). Efficient production of tetraploid melon (Cucumis melo L.) by somatic embryogenesis. *Jap. J. Breed*, 42:137-144.

Fang G and Grumet R (1993). Transformation in Muskmelon (Cucumis Melo L.) in Plant Protoplasts and Genetic Engineering IV Biotechnology in Agriculture and Forestry. Volume 23, pp 209-214.

Fernandez-Silva I, Eduardo I, Blanca J, Esteras C, Pico B, Nuez F, Arus P, Garcia-Mas J, Monforte AJ (2008). Bin mapping of genomic and EST-derived SSRs in melon (Cucumis melo L.). *Theor Appl Genet*, 118(1):139-150.

Ferrari S, Savatin DV, Sicilia F, Gramegna G, Cervone F, De Lorenzo G (2013) Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. *Front Plant Sci*, 13;4:49.

Ferrari S, Sella L, Janni M, De Lorenzo G, Favaron F, D'Ovidio R (2012). Transgenic expression of polygalacturonase-inhibiting proteins in Arabidopsis and wheat increases resistance to the flower pathogen *Fusarium graminearum. Plant Biol*, 14:31–38.

Ficcadenti N, Sestili S, Annibali S, Campanelli G, Belisario A, Maccaroni M, Corazza L (2002). Resistance to *Fusarium oxysporum* f.sp. *melonis* race 1.2 in muskmelon lines Nad-1 and Nad-2. *Plant Dis*, 86:897-900.

Ficcadenti N and Rotino G (1995). Genotype and medium affect shoot re generation of melon. *Plant Cell Tiss Organ Cult*, 40:293-295.

Ficcadenti N, Veronese P, Sestili S, Crino P, Lucretti S, Schiavi M (1995). Influence of genotype on the induction of haploidy in *Cucumis melo* L. by using irradiated pollen. *J Genet Breed*, 49:359–364.

Finco A, Sebastiani MS, Ferrari V, Ficcadenti N (2013). An efficient regeneration protocol for genetic transformation of different melon genotypes (Cucumis melo L. var. cantalupensis). 57th Annual Congress, Italian Society of Agricultural Genetics, Foggia, September 16-19, 1.21.

Galperin M, Patlis L, Ovadia A, Wolf D, Zelcer A, Kenigsbuch D (2003). A melon genotype with superior competence for regeneration and transformation. *Plant Breed*, 122:66–69.

Gao L, JinTu Z, Millett BP, Bradeen JM (2013). Insights into organ-specific pathogen defense responses in plants: RNA-seq analysis of potato tuber *Phytophthora infestans* interactions. *BMC Genomics*, 14:340.

Gao X, Cox KL Jr., He P (2014). Functions of Calcium-Dependent Protein Kinases in Plant Innate Immunity. *Plants*, 3:160-176.

Garcia-Maceira FI, Di Pietro A, Huertas-Gonzalez MD, Ruiz-Roldan MC, Roncero MI (2001). Molecular characterization of an endopolygalacturonase from *Fusarium oxysporum* expressed during early stages of infection. *Applied Environmental Microbiology*, 67:2191-2196.

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G, González VM, Hénaff E, Câmara F, Cozzuto L, Lowy E, Alioto T, Capella-Gutiérrez S, Blanca J, Cañizares J, Ziarsolo P, Gonzalez-Ibeas D, Rodríguez-Moreno L, Droege M, Du L, Alvarez-Tejado M, Lorente-Galdos B, Melé M, Yang L, Weng Y, Navarro A, Marques-Bonet T, Aranda MA, Nuez F, Picó B, Gabaldón T, Roma G, Guigó R, Casacuberta JM, Arús P, Puigdomènech P (2012). The genome of melon (Cucumis melo L.). *PNAS*, 109(29):11872–11877.

Garg R and Jain M (2013). Transcriptome Analyses in Legumes: A Resource for Functional Genomics. *The Plant Genome*, doi: 10.3835/plantgenome2013.04.0011.

Gomez-Gomez L, Bauer Z, Boller T (2001). Both the extracellular leucine-rich repeat domain and the kinase activity of FSL2 are required for flagellin binding and signaling in *Arabidopsis*. *Plant Cell*, 13:1155–63.

Gomez Vazquez J and Tello Marquina JC (2000). Presenzia de la razza 1.2 de *Fusarium oxysporum* f.sp. *melonis* en Almeria. *Boll. San. Veg. Plagas,* 26:27-33.

González M, Xu M, Esteras C, Roig C, Monforte AJ, Troadec C, PujoM, Nuez F, Bendahmane A, Garcia-Mas J, Picó B (2011). Towards a TILLING platform for functional genomics in Piel de Sapo melons. *BMC Res Notes*, 4:289

González VM, Garcia-Mas J, Arús P, Puigdomènech P (2010). Generation of a BAC-based physical map of the melon genome. *BMC Genomics,* 11:339.

Gonzàlez-Fernàndez R, Prats E, Jorrín-Novo JV (2010). Proteomics of Plant Pathogenic Fungi. *Journal of Biomedicine and Biotechnology*, doi:10.1155/2010/932527.

Gonzalez-Ibeas D, Blanca J, Donaire L, Saladié M, Mascarell-Creus A, Cano-Delgado A, Garcia-Mas J, Llave C, Aranda MA (2011). Analysis of the melon (Cucumis melo) small RNAome by high-throughput pyrosequencing. *BMC Genomics*, 12:393.

Gonzalez-Ibeas D, Blanca J, Roig C, Gonzalez-To M, Pico B, TrunigerV, Gomez P, Deleu W, Cano-Delgado A, Arus P, Nuez F, Garcia-Mas J, Puigdomènech P, Aranda MA (2007). MELOGEN: an ESTdatabase for melon functional genomics. *BMC Genomics*, 8:306.

Gonzalo MJ, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arùs P, Monforte AJ (2005). Simple-sequence repeat markers used in merging linkage maps of melon (Cucumis melo L.). *Theor. Appl. Genet.*, 110:802-811.

Gordon TR and Martyn RD (1997). The evolutionary biology of *Fusarium oxysporum. Annu. Rev Phytopathol.*, 35:11-128.

Gostimskiĭ SA, Kokaeva ZG, Konovalov FA (2005). Studying plant genome variation using molecular markers. *Genetika*, 41(4):480-92. [Article in Russian]

Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talón M, Dopazo J, Conesa A (2008). High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Res*, 36:3420–35.

Guis M, Ben-Amor M, Latche A, Peche JC, et al. (2000). A reliable system for the transformation of cantaloupe charentais melon (Cucumis melo L. var. cantaloupensis) leading to a majority of diploid regenerants. *Sci. Hort*, 84:91-99.

Guo Y, Zhao S, Ye F, Sheng Q et al. (2014). Multi Rank Seq: Multiperspective Approach for RNAseq Differential Expression Analysis and Quality Control. *Bio Med Research International*, doi.org/10.1155/2014/248090.

Gusberti M, Gessler C, Broggini G (2013). RNA-Seq Analysis Reveals Candidate Genes for Ontogenic Resistance in Malus - Venturia Pathosystem. *PLoS ONE*, doi:10.1371/journal.pone.0078457.

Haegi A, Catalano V, Luongo L, Vitale S, Scotton M, Ficcadenti N, Belisario A (2013). A newly developed real-time PCR assay for detection and quantification of Fusarium oxysporum and its use in compatible and incompatible interactions with grafted melon genotypes. *Phytopathology*, 103:802–810.

Hammond-Kosack KE and Jones JD (1997). Plant disease resistance genes. *Annu Rev Plant Physiol Plant Mol Biol*., 48:575-607.

Harel-Beja R, Tzuri G, Portnoy V, Lotan-Pompan M, Lev S, Cohen S, Dai N, Yeselson L, Meir A, Libhaber SE, Avisar E, Melame T, van Koert P, Verbakel H, Hofstede R, Volpin H, Oliver M, Fougedoire A, Stalh C, Fauve J, Copes B, Fei Z, Giovannoni J, Ori N, Lewinsohn E, Sherman A, Burger J, Tadmor Y, Schaffer AA, Katzir N (2010). A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. *Theor Appl Genet*, 121(3):511-33.

Herman R and Perl-Treves R (2007). Characterization and Inheritance of a new source of resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in *Cucumis melo*. *Plant Disease*, 91 (9) doi:10.1094/PDIS-91-9-1180.

Herman R, Zvirin Z, Kovalski I, Freeman S, Denisov Y, Zuri G, Katzir N, Perl-Treves R (2008). Characterization of Fusarium race 1.2 resistance in melon and mapping of major QTL for this trait near a fruit-netting locus. Proceedings of the IX Eucarpia meeting on genetics and breeding of Cucurbitaceae Avignon (France).

Inoue I, Namiki F, Tsuge T (2002). Plant colonization by vascular wilt fungus Fusarium oxysporum requires FOW1, a gene encoding a mitochondrial protein. *The Plant Cell*, 14:1869-1883.

Jain R, Sinha A, Jain D, Kachhwaha S, Kothari SL (2011). Adventitious shoot regeneration and *in vitro* biosynthesis of steroidal lactones in *Withania coagulans* (stock) Dunal. *Plant Cell Tissue Organ Cult*, 105: 135-140.

Jakubowiczm M and Nowak W (2010). 1-Aminocyclopropane-1-Carboxylate Synthase, an enzyme of Ethylene biosynthesis. In: Comprehensive natural products II: chemistry and biology. *Townsend CA and Ebizuka Y, Eds,* (Elsevier press). Volume 10 p. 110.

Jayanthi M and Mandal PK (2001). Plant regeneration through somatic embryogenesis and RAPD analysis of regenerated plants in *Tylophora indica* (Burm. F. Merrill.). *In Vitro Cell. Dev. Biol.-Plant*, 37:576-580.

Jefferson R, Kavanagh A, Bevan M (1987). GUS fusions: *ß-glucoronidase* as fusion marker in higher plants. *EMBO J*, 6:3901-3907.

Jikku J (2013). Studies on the induction of variation through in vitro culture in *Jatropha curcas* L. *Ph.D. thesis dissertation.*

Jin S, Mushke R, Zhu H, Tu L, Lin Z, Zhang Y, Zhang X (2008). Detection of somaclonal variation of cotton (Gossypium hirsutum) using cytogenetics, flow cytometry and molecular markers. *Plant Cell Rep*, 27:1303–1316.

Jones JDG and Dangl JL (2006). The plant immune system. *Nature*, 444(16):323-329.

Jones DA and Takemoto D (2004). Plant innate immunity - direct and indirect recognition of general and specific pathogen-associated molecules. *Curr Opin Immunol.*, 16(1):48-62.

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *PNAS,* 103:11086–11091.

Kathal R, Bhatnagar SP, Bhojwani SS (1986). Regeneration of shoots from hypocotyl callus of *Cucumis melo* cv. Pusa sharbati. *Journal of Plant Physiology,* 126:59–62.

Kazan K and Manners JM (2009). Linking development to defense: auxin in plant–pathogen interactions. *Trends in Plant Science,* 14: 373–382.

Kempela A, Schädlerb M, Chrobocka T, Fischera M, van Kleunena M (2011).Tradeoffs associated with constitutive and induced plant resistance against herbivory, *PNAS,* 108(14):5685–5689.

Keng CL and Hoong LK (2006). In vitro plantlets regeneration from nodal segments of muskmelon (Cucumis melo L.). *Biotechnol,* 4:354.

Kidd BN, Kadoo NY, Dombrecht B, Tekeoğlu M, Gardiner DM, Thatcher LF, Aitken EAB, Schenk PM, Manners JM, Kazan K (2011). Auxin Signaling and Transport Promote Susceptibility to the Root-Infecting Fungal Pathogen *Fusarium oxysporum* in Arabidopsis. *MPMI,* 24, 6-733–748.

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol,* 14:R36.

Kombrink A, Sánchez-Vallet A, Thomma BP (2011). The role of chitin detection in plant - pathogen interactions. *Microbes Infect*, 13(14-15):1168-76.

Kumar N, Vijayanand KG, Reddy MP (2011). Plant regenerationin non-toxic *Jatropha curcas* - impacts of plant growth regulators, source and type of explants. *Journal of Plant Biochemistry and Biotechnology*, 20:125-133.

Łabaj PP, Leparc GG, Linggi BE, Markillie LM, Wiley HS, Kreil DP (2011). Characterization and improvement of RNA-Seq precision in quantitative transcript expression profiling. *Bioinformatics*, 27(13):i383-91.

Lakshmanan V, Venkataramareddy SR, Neelwarne B (2007). Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. *Electron J Biotechnol,* 10:106–113.

Lam ENK and Lawton M (2001). Programmed cell death, mitochondria and the plant hypersensitive response. *Nature*, 411:848-853.

Larkin PJ and Scowcroft WR (1981). Somaclonal variation-a novel source of variability from plant cell cultures for plant improvement. *Theor Appl Genet,* 60:197-214.

Latge JP and Beauvais A (2014). Functional duality of the cell wall. *Curr Opin Microbiol,* 20:111–7.

Łazniewska J, Macioszek VK, Lawrence CB, Kononowicz AK (2010). Fight to the death: Arabidopsis thaliana defense response to fungal necrotrophic pathogens. *Acta Physiologiae Plantarum,* 32:1–10.

Lehmann P (2002). Structure and evolution of plant disease resistance genes. *J. Appl. Genet,* 43(4):403-414.

Lehtonen MT, Akita M, Frank W, Reski R, Valkonen JPT (2011). Involvement of a class III peroxidase and the mitochondrial protein TSPO in oxidative burst upon treatment of moss plants witha fungal elicitor. *Mol Plant Microbe Interact,* 25:363–371.

Li CY, Deng GM, Yang J, Viljoen A, Jin Y, Kuang RB et al. (2012). Transcriptome profiling of resistant and susceptible Cavendish banana roots following inoculation with *Fusarium oxysporum* f. sp. *cubense* tropical race 4. *BMC Genomics*, 13(1):374.

Li H, Zhou SY, Zhao WS, Su SC, Peng YL (2009). A novel wall-associated receptor-like protein kinase gene, OsWAK1, plays important roles in rice blast disease resistance. *Plant Molecular Biology,* 69: 337–346.

Liu X, Grabherr HM, Willmann R, et al. (2014). Host-induced bacterial cell wall decomposition mediates pattern-triggered immunity in Arabidopsis. *eLife,* 3:e01990

Liu X, Yang L, Zhou X, Zhou M, Lu Y, Ma L, Ma H, Zhang Z (2013). Transgenic wheat expressing *Thinopyrum intermedium* MYB transcription factor TiMYB2R-1 shows enhanced resistance to the take-all disease. *Journal of Experimental Botany,* 64(8):2243–2253.

Llorente F, Muskettb P, Sanchez-Valleta A, Lopeza G, Ramosa B, Sanchez-Rodrıgueza C, Jorda L, Parkerb J, Molina A (2008). Repression of the Auxin Response Pathway Increases Arabidopsis Susceptibility to Necrotrophic Fungi. Molecular Plant, 1(3):496-509.

Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R (2003). Ethylene response factor1 integrates signals from ethylene and jasmonate pathways in plant defense. *Plant Cell*, (1):165-78.

Lu H, Rate DN, Tae Song J, Greenberg JT (2003). ACD6, a Novel Ankyrin Protein, Is a Regulator and an Effector of Salicylic Acid Signaling in the Arabidopsis Defense Response. *The Plant Cell*, 15, 2408–2420.

Luo W and Brouwer C (2013). Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics,* 29(14): 1830-1831.

Luongo L, Ferrarini A, Haegi A, Vitale S, Polverari A, Belisario A (2014). Genetic Diversity and Pathogenicity of *Fusarium oxysporum* f.sp. *melonis* Races from Different Areas of Italy. *J Phytopathol*, doi: 10.1111/jph.12280.

Ma L, Cornelissen BJC, Takken FLW (2013). A nuclear localization for Avr2 from *Fusarium oxysporum* is required to activate the tomato resistance protein I-2. *Frontiers in Plant science,* 4:94.

Ma LJ, Geiser DM, Proctor RH, Rooney AP, O'Donnell K, Trail F, Gardiner DM, Manners JM, Kazan K (2013). Fusarium Pathogenomics. *Annu. Rev. Microbiol.,* 67:399–416.

Ma W and Berkowitz GA (2011). Ca2+ conduction by plant cyclic nucleotide gated channels and associated signaling components in pathogen defense signal transduction cascades. *New Phytologist,* 190:566–572.

Madrid MP, Di Pietro A, Roncero MI (2003). Class V chitin synthase determines pathogenesis in the vascular wilt fungus *Fusarium oxysporum* and mediates resistance to plant defense compounds. *Mol Microbiology,* 46:257-266.

Mallon R, Rodriguez-Oubina J, Luz Gonzalez M (2010). *In vitro* propagation of the endangered plant *Centaurea ultreiae*: assessment of genetic stability by cytological studies, flow cytometry and RAPD analysis. *Plant Cell Tissue Organ Cult,* 101:31–39.

Mallick MFR and Masui M (1986). Origin, distribution and taxonomy of melons. *Scientia Hort., 28: 251-261.*

Manniche L (1989). An ancient Egyptian Herbal. British Museum Press, London.

Maor R, Esky MP, Horwitz BA, Sharon A (1998). Use of green fluorescent protein (GFP) for studying development and fungal-plant interaction in *Cochliobolus heterostrophus. Mycol. Res*, 102(4):491–496

Martin LBB, Fei Z, Giovannoni JJ, Rose JKC (2013). Catalyzing plant science research with RNA-seq. *Frontiers in Plant Science,* 4:66.

Martin M (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal,* 17:10–12.

Martínez-Gómez Pedro, Sánchez-Pérez Raquel, Rubio Manuel (2012). Clarifying omics concepts, challenges, and opportunities for Prunus breeding in the postgenomic era. *OMICS,* 16(5):268-83.

Marukawa S (1979). Studies on varieties of Cucurbita spp. As rootstocks for cucurbitaceous vegetables, with special reference to their grafting compatibility. Bull. Ibaraki-Ken Hortic. Exp. Stn. Special Issue, n°.5

Mascarell-Creus A, Canizares J, Vilarrasa-Blasi J, Mora-Garcia S, Blanca J, Gonzalez-Ibeas D, Saladié M, Roig C, Deleu W, Picó-Silvent B, López-Bigas N, Aranda MA, Garcia-Mas J, Nuez F, Puigdomènech P, Caño-Delgado A (2009). An oligo-based microarray offers novel transcriptomic approaches for the analysis of pathogen resistance and fruit quality traits in melon (Cucumis melo L.). *BMC Genomics,* 10:467.

Mehta R, Sharma V, Sood A, Sharma M, Sharma RK (2011). Induction of somatic embryogenesis and analysis of genetic fidelity of in vitro-derived plantlets of *Bambusa nutans* Wall., using AFLP markers. *Eur J Forest Res,* 130:729–736.

Meinhardt LW, Costa GG, Thomazella DP, Teixeira PJ, Carazzolle MF, Schuster SC, Carlson JE, Guiltinan MJ, Mieczkowski P, Farmer A, Ramaraj T, Crozier J, Davis RE, Shao J, Melnick RL, Pereira GA, Bailey BA (2014). Genome and secretome analysis of the hemibiotrophic fungal pathogen, *Moniliophthora roreri*, which causes frosty pod rot disease of cacao: mechanisms of the biotrophic and necrotrophic phases. *BMC Genomics,* doi: 10.1186/1471-2164-15-164.

Mengiste T, Chen X, Salmeron J, Dietrichb R (2003). The Botrytis susceptible1 Gene Encodes an R2R3MYB Transcription Factor Protein That Is Required for Biotic and Abiotic Stress Responses in Arabidopsis. *Plant Cell,* 15(11): 2551–2565.

Michielse CB and Rep M (2009). Pathogen profile update: Fusarium oxysporum. *Mol. Plant Pathol*, 10:311–324.

Möller B and Weijers D (2009). Auxin Control of Embryo Patterning. *Cold Spring Harb Perspect Biol*, 1:a001545.

Monforte AJ, Oliver M, Gonzalo MJ, Alvarez JM, Dolcet-Sanjuan R, Arus P (2004). Identification of quantitative trait loci involved in fruit quality traits in melon (Cucumis melo L.). *Theor. Appl. Genet,* 108:750-758.

Moon JG, Choo BK, Hs D, Kwon TH, Yang MS, Ryu JH (2000). Effects of growth regulators on plant regeneration from the cotyledon explant in oriental melon (Cucumis melo L.). *Kor J Plant Tissue Cult,* 27:1–6.

Moreno V, Garcia-Sogo M, Granell I, Garcia-Sogo B, Roig LA (1985). Plant regeneration from calli of melon (Cucumis melo L., cv. Amarillo Oro). *Plant Cell Tissue Organ Cult,* 5:139-146.

Mur LAJ, Carver TLW, Prats E (2006). NO way to live; the various roles of nitric oxide in plant pathogen interactions. *J. Exp. Bot,* 57:489–505.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant,* 15:473-497.

Muto H, Yabe N, Asami T, Hasunuma K, Yamamoto KT (2004). Overexpression of constitutive differential growth 1 gene, which encodes a RLCKVII - subfamily protein kinase, causes abnormal differential and elongation growth after organ differentiation in Arabidopsis. *Plant Physiol,* 136:3124–3133.

Nadha HK, Kumar R, Sharma RK, Anand M, Sood A (2011). Evaluation of clonal fidelity of in vitro raised plants of *Guadua angustifolia* Kunth using DNA-based markers. *J Med Plants Res,* 5:563–5641.

Namiki F, Shiomi T, Nishi K, Kayamura T, Tsuge T (1998). Pathogenicity and genetic variation in the Japanese strains of *Fusarium oxysporum* f. sp. *melonis. Phytopathology*, 88:804-810.

Narayanasamy P (2008). Molecular Biology in Plant Pathogenesis and Disease Management: Disease. *Springer Science & Business Media,* Volume 3.

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD (2006). A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science,* 312:436-439.

Nemchinov LG, Shabala L, Shabala S (2008). Calcium Efflux as a Component of the Hypersensitive Response of *Nicotiana benthamiana* to *Pseudomonas syringae*. *Plant Cell Physiol,* 49(1):40–46.

Ngezahayo F, Dong Y, Liu B (2007). Somaclonal variation at the nucleotide sequence level in rice (Oryza sativa L.) as revealed by RAPD and ISSR markers, and by pairwise sequence analysis. *Journal of Applied Genetics,* 48(4):329-336.

Niedz RP, Smith SS, Dunbar KV, Stephens CT, Murakishi HH (1989). Factors affecting shoot regeneration from cotyledonary explants of *Cucumis melo*. *Plant Cell Tissue and Organ Culture,* 18:313–319.

Nieto C, Piron F, Dalmais M, Marco CF, Moriones E, Gómez-Guillamón ML, Truniger V, Gómez P, Garcia-Mas J, Aranda MA, Bendahmane A (2007). EcoTILLING for the identification of allelic variants of melon eIF4E, a factor that controls virus susceptibility. *BMC Plant Biol,* 7:34.

Nora FR, Peters JA, Schuch MW, Lucchetta L, Marini L, Silva, JA, Rombaldi CV (2012). Melon regeneration and transformation using an apple ACCoxidase antisense gene. *Rev. Bras. de Agrociência,* 7(3):201-204.

Norelli JL and Aldwinckle HS (1993). The role of aminoglycoside antibiotics in the regeneration and selection of neomycin phosphotransferase transgenic apple tissue. *J. Amer. Soc. Hort. Sci*., 118:311–316.

Ntui VO, Thirukkumaran G, Iioka S, Mii M (2009). Efficient plant regeneration via organogenesis in "Egusi" melon (Colocynthis citrullus L.). *Scientia Horticulturae,* 119:397–402.

Nunez-Palenius HG, Gomez-Lim M, Ochoa-Alejo N, Grumet R, et al. (2008). Melon fruits: genetic diversity, physiology, and biotechnology features. *Crit. Rev. Biotechnol,* 28:13-55.

Nunez-Palenius HG, Cantliffe DJ, Huber DJ, Ciardi J, et al. (2006). Transformation of a muskmelon "Galia" hybrid parental line (Cucumis melo L. var. reticulatus Ser.) with an antisense ACC oxidase gene. *Plant Cell Rep,* 25:198-205.

Nürnberger T, Brunner F, Kemmerling B, Piater L (2004). Innate immunity in plants and animals: striking similarities and obvious differences. *Immunol Rev,* 198**:**249–266.

Nürnberger T and Brunner F (2002). Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. *Curr Opin Plant Biol*, 5, 318–324.

Ophir R, Eshed R, Harel-Beja R, Tzuri G, Portnoy V, Burger Y, Uliel S, Katzir N, Sherman A (2010). High-throughput marker discovery in melon using a self-designed oligo microarray. *BMC Genomics,* 11:269.

Osakabe Y, Osakabe K, Shinozaki K, Tran Lam SP (2014). Response of plants to water stress. *Frontiers in Plant Science,* doi: 10.3389/fpls.2014.00086.

Oshlack A and Wakefield MJ (2009). Transcript length bias in RNA-seq data confounds systems biology. *Biology direct,* 4:14.

Oumouloud A, El-Otmani M, Chikh-Rouhou H, Garces Claver A, Gonzalez Torres R, Perl-Treves R, Alvarez JM (2013). Breeding melon for resistance to Fusarium wilt: recent developments. *Euphytica,* 192:155–169.

Oumouloud A, Arnedo-Andrès MS, Gonzàlez-Torres R, Alvarez JM (2010). Inheritance of resistance to *Fusarium oxysporum* f. sp. *melonis* races 0 and 2 in melon accession Tortuga. *Euphytica*, 176:183–189.

Oumouloud A, Arnedo-Andrès MS, Gonzàlez-Torres R, Alvarez JM (2009). Morphological and molecular characterization of melon accessions resistant to Fusarium wilts. *Euphytica*, 169:69–79.

Ozsolak F and Milos PM (2010). RNA sequencing: advances, challenges and opportunities. *Nat Rev Genet.*, 12(2):87-98.

Palmer GE (2011). *Commun Integr Biol*, 4(2): 240–242)Pandey RN, Singh SP, Rastogi J, Sharma ML, Singh RK (2012). Early assessment of genetic fidelity in sugarcane (Saccharum officinarum) plantlets regenerated through direct organogenesis with RAPD and SSR markers. *Aust J Crop Sci*, 6:618–624.

Pangalo KJ (1929). Critical review of the main literature on the taxonomy, geography and origin of cultivated and partially wild melons. *Trudy Prikl. Bot*., 23:397-442 (In Russian, and translated into English for USDA by G. Saad in 1986).

Paris MK, Zalapa JE, McCreight JD, Staub JE (2008). Genetic dissection of fruit quality components in melon (Cucumis melo L.) using a RIL population derived from exotic elite US western shipping germplasm. *Mol. Breed*, 22:405–419.

Pech JC, Bernadac A, Bouzayen M, Latche A, Dogimont C, Pitrat M (2007). Melon Biotechnology in Agriculture and Forestry. In: Pua EC, Davey MR (Eds) Transgenic Crops V. vol 60. Chap I.9, pp 209–240.

Peng Y, Bartley LE, Canlas P, Ronald PC (2010). OsWRKY IIa Transcription Factors Modulate Rice Innate Immunity. *Rice*, 3: 36–42.

Perchepied L, Dogimont C, Pitrat M (2005). Strain-specific and recessive QTLs involved in the control of partial resistance to *Fusarium oxysporum* f.sp. *melonis* race 1.2 in a recombinant inbred line population in melon. *Theor Appl Genet*, 111:65-74.

Perchepied L and M. Pitrat (2004). Polygenic inheritance of partial resistance to *Fusarium oxysporum* f.sp. *melonis* race 1.2 in melon. Phytopathology, 94(12): 1331-1336

Périn C, Hagen LS, de Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002). A reference map of *Cucumis melo* based on two recombinant inbred line populations. *Theor. Appl. Genet*, 104:1017-1034.

Petri C, Wang H, Alburquerque N, Faize M, Burgos L (2008). Agrobacterium-mediated transformation of apricot (Prunus armeniaca L.) leaf explants. *Plant Cell Rep*, 27(8):1317-24.

Pieterse CMJ, Leon-Reyes A, van der Does D, Verhage A, Annemart K, van Pelt JA, van Wees SCM (2012). Networking by small-molecule hormones in plant immunity induced resistance in plants against insects and diseases. *IOBC-WPRS Bulletin*, 83:77-80.

Pitrat M (2008). Melon. In: Prohens J and Nuez F (eds.) Handbook of plant breeding. Vegetables I. Asteraceae, Brassicaceae, Chenopoidicaceae, and Cucurbitaceae, Springer, USA, pp. 283–315.

Popescu SC, Popescu GV, Bachan S, Zhang Z, Seay M, Gerstein M, Snyder M, Dinesh-Kumar SP (2007). Differential binding of calmodulin-related proteins to their targets revealed through high-density Arabidopsis protein microarrays. PNAS, 104:4730–4735.

Portnoy V, Diber A, Pollock S, Karchi H, Lev S, Tzuri G, Harel-Beja R, Forer R, Portnoy VH, Lewinsohn E, Tadmor Y, Burger J, Schaffer A, Katzir N (2011). Use of Non-Normalized, Non-Amplified cDNA for 454-Based RNA Sequencing of Fleshy Melon Fruit. *The Plant Genome*, 4:36–46.

Prados-Rosales RC, Roldán-Rodríguez R, Serena C, López-Berges MS, Guarro J, Martínez-del-Pozo Á, Di Pietro A (2012). A PR-1-like Protein of *Fusarium oxysporum* Functions in Virulence on Mammalian Hosts. *J Biol Chem*, 287(26): 21970–21979.

Qi L, Yan J, Li Y, Jiang H, Sun J et al. (2012). *Arabidopsis thaliana* plants differentially modulate auxin biosynthesis and transport during defense responses to the necrotrophic pathogen *Alternaria brassicicola*. *New Phytologist*, 195: 872–882.

Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010). Characterization of the defense transcriptome responsive to *Fusarium oxysporum* infection in Arabidopsis using RNAseq. *Trends Plant Sci*, 15(7):395-401.

Rani V, Raina S (2000). Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. *In Vitro Cell Dev Biol-Plant*, 36:319–330.

Rani V, Parida A, Raina S (1995). Random amplified polymorphicDNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoids* Marsh. *Plant Cell Rep*, 14:459–462.

Reddy ASN, Ali GS, Celesnik H, Day IS (2011). Coping with stresses: Roles of calcium- and calcium/calmodulin-regulated gene expression. Plant Cell, 23:2010–2032.

Ren Y, Bang H, Gould J, Rathore K, Patil B, Crosby K (2013). Shoot regeneration and ploidy variation in tissue culture of honeydew melon (Cucumis melo L. inodorus). *In Vitro Cellular & Developmental Biology - Plant*, 49(2):223.

Renner SS, Schaefer H, Kocyan A (2007). Phylogenetics of Cucumis (Cucurbitaceae): cucumber (C. sativus) belongs in an Asian/Australian clade far from melon (C. melo). *BMC Evol Biol*, 10(7):58.

Risser G, Banihashemi Z, Davis DW (1976). A proposed nomenclature of *Fusarium oxysporum* f. sp. *melonis* races and resistance genes in *Cucumis melo*. *Phytopathology*, 66:1105-1106.

Rodriguez-Gàlvez E and Mendgen K (1995). The infection process of Fusarium oxysporum in cotton root tips. *Protoplasma*, 189:61-72.

Robinson RW and Decker-Walters DS (1997). Cucurbits. CAB International, Wallingford, UK. ISBN: 0 85199 133 5.

Robinson MD and Oshlack A (2010). A scaling normalization method for differential expression analysis of RNA-seq data. *Genome biology*, 11:R25.

Rovenich H, Boshoven JC, Thomma BPHJ (2014). Filamentous pathogen effector functions: of pathogens, hosts and microbiomes. *Current Opinion in Plant Biology*, 20:96-103.

Roy AR, Sajeev S, Pattanayak A, Deka BC (2012). TDZ induced micropropagation in *Cymbidium giganteum* Wall. Ex Lindl. and assessment of genetic variation in the regenerated plants. *Plant Growth Regul*, 68:435–445.

Rubio M, Rodríguez-Moreno L, Ballester AR, de Moura MC, Bonghi C, Candresse T, Martínez-Gómez P (2014). Analysis of gene expression changes in peach leaves in response to Plum pox virus infection using RNA-Seq. *Mol Plant Pathol*, doi: 10.1111/mpp.12169.

Ruiz-Roldán MC, Köhli M, Roncero MIG, Philippsen P, Di Pietro A, Espeso EA (2010). Nuclear Dynamics during Germination, Conidiation, and Hyphal Fusion of *Fusarium oxysporum*. *Eukaryot Cell*, 9(8):1216–1224.

Sahijram L, Soneji JR, Bollamma KT (2003). Analyzing somaclonal variation in micropropagated bananas (Musa spp.). *In vitro Cell. Dev. Biol. - Plant*, 39:551-556.

Schreuder W, Lamprecht SC, Holz G (2000). Race determination and vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *melonis* from South Africa. *Plant Dis*, 84:231-234.

Sebastiani MS, Bagnaresi P, Sestili S, Biselli C, Orrù L, Ferrari V, De Lorenzo G, Ficcadenti N (2014). Exploring resistance response in melon-*Fusarium oxysporum* f.sp. *melonis* race 1.2 interaction using high throughput RNA sequencing. Abstract Book – Poster presentation, Plant Biology Europe FESPB/EPSO Congress, Dublin, June 22-26, p. 435.

Sebastiani MS and Ficcadenti N (2013). An efficient protocol for in vitro plant regeneration from cotyledon explants of different melon genotypes (Cucumis melo L. var. cantalupensis). Abstract book - Young Researches in Life Science (YRLS) Conference, Paris, May 22-24, p.129.

Sedgwick SG and Smerdon SJ (1999). The ankyrin repeat: a diversity of interactions on a common structural framework. *Trends Biochem Sci*, 24(8):311-6.

Seo PJ and Park CM (2010). MYB96-mediated abscisic acidsignals induce pathogen resistance response by promoting salicylicacid biosynthesis in Arabidopsis. *New Phytol*, 186:471–483.

Sestili S, Sebastiani MS, Belisario A, Ficcadenti N (2014). Reference gene selection for gene expression analysis in melon infected by F*usarium oxysporum* f.sp. *melonis. J. Plant Biochem. Biotechnol*, 23(3):238-248.

Sestili S, Polverari A, Luongo L, Ferrarini A, Scotton M, Hussain J, Delledonne M, Ficcadenti N, Belisario A (2011). Distinct colonization patterns and cDNA-AFLP transcriptome profiles in compatible and incompatible interactions between melon and different races of *Fusarium oxysporum* f. sp. *melonis*. *BMC Genomics*, 12:122.

Sestili S, Campanelli G, Ferrari V, Belisario A, Papa R, Ficcadenti N (2005). Development of molecular markers linked to the resistance towards *Fusarium oxysporum* f.sp. *melonis* race 1.2w in melon. XLIX SIGA, Potenza, 12-15 Settembre

Sheidai M, Aminpoor H, Noormohammadi Z, Farahani F (2008). RAPD analysis of somaclonal variation in banana (Musa acuminata L.) cultivar Valery. *Acta Biologica Szegediensis*, 52(2):307-311.

Singh P and Zimmerli L (2013). Lectin receptor kinases in plant innate immunity. *Frontier in Plant Sciences,* doi: 10.3389/fpls.2013.00124.

Skadsen, RW and Hohn TA (2004). Use of *Fusarium graminearum* transformed with *gfp* to follow infection patterns in barley and Arabidopsis. *Physiol Mol Plant Pathol,* 64,45–53.

Skoog F and Miller C (1956). Chemical regulation of growth and organ formation in plant tissues cultivated in vitro. *Symposium of the Society of Experimental Botany,* 9:118-131.

Stec B (2006). Review Plant thionins – the structural perspective. *Cell. Mol. Life Sci,* 63:1370–1385.

Stegniĭ VN, Chudinova IV, Salina EA (2000). RAPD analysis of the flax (Linum usitatissimum L.) varieties and hybrids of various productivity. *Genetika,* 36(10):1370-3. [Article in Russian]

Stotz HU, Thomson JG, Wang Y (2009). Plant defensins: Defense, development and application. *Plant Signaling & Behavior,* 4(11):1010-1012.

Su YH, Liu YB, Zhang XS (2011). Auxin–Cytokinin Interaction Regulates Meristem Development Molecular Plant, 4(4):616–625.

Sultana RS and Mahabubur RMd (2014). Melon crops improvement through biotechnological techniques for the changing climatic conditions of the 21st century. *International Journal of Genetics and Genomics,* 2(3): 30-41.

Sutherland R, Viljoen A, Myburg AA, van den Berg N (2012). *Pathogenicity genes in Fusarium oxysporum f. sp. cubense* race 4. *South African Journal of Science,* Volume 109|Number 5/6.

Staskawicz BJ (2001). Genetics of Plant-Pathogen Interactions Specifying Plant Disease Resistance. *Plant Physiol*, 125:73-76

Swarupa V, Ravishankar KV, Rekha A (2014). Plant defense response against *Fusarium oxysporum* and strategies to develop tolerant genotypes in banana. *Planta*, 239:735–751.

Takken F and Rep M (2010). The arms between tomato and *Fusarium oxysporum*. *Mol Plant Pathol,* 11:309-314.

Taler D, Galperin M, Benjamin I, Cohen Y, Kenigsbuch D (2004). Plant enzymatic resistance (eR) genes encoding for photorespiratory enzymes confer resistance against disease. *Plant Cell,* 16:172–184.

Thiruvengadam M, Rekha KT, Jayabalan N, Yang CH, Chung IM (2010). High frequency shoot regeneration from leaf explants through organogenesis of bitter melon (Momordica charantia L.) *Plant Biotechnol Rep,* 4:321-328.

Thomma BPHJ, Nürnberger T, Joostena MHAJ (2011). The Blurred PTI-ETI dichotomy of PAMPs and Effectors. Plant Cell, 23(1):4–15.

Trapnell C, Roberts A, Goff L, Pertea G, Kim D, et al. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols*, 7:562–578.

Truman W, Bennett MH, Kubigsteltig I, Turnbull C, Grant M (2007). Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. *PNAS,* 104(3):1075-80.

Tucker SL and Talbot NJ (2001). Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annu. Rev. Phytopathol.,* 39:385–417.

Upadhyay S and Shaw BD (2008). The Role of actin, fimbrin, and endocytosis in growth of hyphae in *Aspergillus nidulans*. *Molecular Microbiology*, 68(3):690–705).

Valente MT, Infantino A, Aragona M (2011). Molecular and functional characterization of an endoglucanase in the phytopathogenic fungus *Pyrenochaeta lycopersici. Current Genetics,* 57(4):241-251.

van Leeuwen H, Monfort A, Zhang HB, Puigdomènech P (2003). Identification and characterization of a melon genomic region containing a resistance gene cluster from a constructed BAC library. Microlinearity between *Cucumis melo* and *Arabidopsis thaliana*. *Plant Mol Biol,* 51:703-718.

Van Loon LC, Rep M, Pieterse CMJ (2006). Significance of inducible defense - related proteins in infected plants. *Annu. Rev.Phytopathol*, 44:135–162.

Vartapetian AB, Tuzhikov AI, Chichkova NV, Taliansky M, Wolpert TJ (2011). A plant alternative to animal caspases: subtilisin-like proteases. *Cell Death and Differentiation*, 18:1289–1297.

Veronese P, Ruiz MT, Coca MA, Hernandez-Lopez A, Lee H, Ibeas JI, Damsz B, Pardo JM, Hasegawa PM,. Bressan RA, Narasimhan Meena L. (2003). In Defense against Pathogens. Both Plant Sentinels and Foot Soldiers Need to Know the Enemy. *Plant Physiol,* doi/10.1104/pp.102.013417.

Vilaça de Vasconcelos MJ, Schusterschitz Antunes M, Marques Barbosa S, Siqueira de Carvalho CH (2008). RAPD analysis of callus regenerated and seed grownplants of maize (Zea mays L.). *Rev Brasileira de Milho e Sorgo*, 7:93-104.

Walters D and Heil M (2007). Costs and trade-offs associated with induced resistance. *Physiol. Mol. Plant Pathol,* 71, 3-17.

Wan J, Zhang XC, Neece D, et al. (2008). A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. *Plant Cell*, 20:471–81.

Wang Z, Jia C, Li J, Huang S, Xu B, Jin Z (2014). Activation of salicylic acid metabolism and signal transduction can enhance resistance to Fusarium wilt in banana (Musa acuminata L. AAA group, cv. Cavendish). *Funct Integr Genomics,* doi 10.1007/s10142-014-0402-3.

Wang Y, Liu TB, Patel S, Jiang L, Xue C (2011). The casein kinase I protein Cck1 regulates multiple signaling pathways and is essential for cell integrity and fungal virulence in *Cryptococcus neoformans*. *Eukaryot Cell,* 10(11):1455-64.

Wang CI, Guncar G, Forwood JK, Teh T, Catanzariti AM, Lawrence GJ, Loughlin FE, Mackay JP, Schirra HJ, Anderson PA, Ellis JG, Dodds PN, Kobe B (2007). Crystal structures of flax rust

avirulence proteins AvrL567-A and -D reveal details of the structural basis for flax disease resistance specificity. *Plant Cell,* 19:2898–2912.

Willmann R, Lajunen HM, Erbs G, et al. (2011). Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *PNAS,* 108:19824–9.

Wrzaczek M, Brosche M, Salojarvi J, Kangasjarvi S, Idanheimo N, Mersmann S, Robatzek S, Karpiński S, Karpińska B, Kangasjarvi J (2010). Transcriptional regulation of the CRK/DUF26 group of Receptor-like protein kinases by ozone and plant hormones in Arabidopsis. *BMC Plant Biology,* 10:95.

Xin M, Wang X, Peng H, Yao Y, Xie C, Han Y, Ni Z, Sun Q (2012). Transcriptome Comparison of Susceptible and Resistant Wheat in Response to Powdery Mildew Infection. *Genomics Proteomics Bioinformatics*, 10:94–106.

Yadeta KA and Thomma BPHJ (2013). The xylem as battleground for plant hosts and vascular wilt pathogens. *Front Plant Sci,* 4:97.

Yalcin-Mendi NY, Ipek M, Serbest-Kobaner S, Curuk S, Aka Kacar Y, Cetiner S, Gaba V, Grumet R (2004). *Agrobacterium* mediated transformation of Kirkagac 637 a recalcitrant melon (Cucumis melo) cultivar with ZYMV coat protein encoding gene. *Eur J Hortic Sci*, 69:258–262.

Yang T, Chaudhuri S, Yang L, Chen Y, Poovaiah BW (2004). Calcium/calmodulin up-regulates a cytoplasmic receptor-like kinase in plants. *J. Biol. Chem,* 279:42552–42559.

Yang X, Deng F, Ramonell KM (2012). Receptor-like kinases and receptor-like proteins: keys to pathogen recognition and defense signaling in plant innate immunity. *Frontiers in Biology*, 7:155–166.

Yang ZB, You JF, Yang ZM (2007). Manganese uptake and transportation as well as antioxidant response to excess manganese in plants. PMID: 18349501; 33(6):480-8. [Article in Chinese]

Young MD, Wakefield MJ, Smyth GK, Oshlack A (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome biology,* 11:R14.

Zhang H, Yang Y, Wang C, Liu M, Li H, Fu Y, Wang Y, Nie Y, Liu X, Ji W (2014). Large-scale transcriptome comparison reveals distinct gene activations in wheat responding to stripe rust and powdery mildew. *BMC Genomics,* 15:898.

Zhang H, Peng G, Feishi L (2011). Efficient plant regeneration from cotyledonary node explants of *Cucumis melo* L. *Afr J Biotechnol,* 10(35):6757–6761.

Zink FW (1992). Genetics of resistance to *Fusarium oxysporum* f. sp. *melon*is races 0 and 2 in muskmelon cultivars Honey Dew, Iroquois, and Delicious 51. *Plant Disease,* 76:162-166.

Zipfel C, Kunze G, Chinchilla D, et al. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium* mediated transformation. *Cell,*125:749–60.

Zhu QH, Stephen S, Kazan K, Jin G, Fan L, Taylor J, Dennis ES, Helliwell CA, Wang MB (2013). Characterization of the defense transcriptome responsive to *Fusarium oxysporum*-infection in Arabidopsis using RNA-seq. *Gene,* 512:259–266.

Zhu S, Dai YM, Zhang XY, Ye JR, Wang MX, Huang MR (2013). Untangling the transcriptome from fungus-infected plant tissues. Gene, 519 238–244.

Zouari I, Salvioli A, Chialva M, Novero M, Miozzi L, Tenore GC, Bagnaresi P, Bonfante P (2014). From root to fruit: RNA-Seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. *BMC Genomics*, 15:221.

Zuniga TL, Zitter TA, Gordon TR, Schroeder DT, Okamoto D (1997). Characterization of pathogenic races of *Fusarium oxysporum* f. sp. *melonis* causing Fusarium wilt of melon in New York. *Plant Dis*, 81:592-596.

Zvirin T, Herman R, Brotman Y, Denisov Y, Belausov E, Freeman S, Perl-Treves R (2010). Differential colonization and defense responses of resistant and susceptible melon lines infected by *Fusarium oxysporum* race1.2. *Plant Pathol*, 59:576–585.

7. LIST OF PUBLICATIONS

1 - Sestili S, Sebastiani MS, Belisario A, Ficcadenti N (2014). Reference gene selection for gene expression analysis in melon infected by *Fusarium oxysporum* f.sp. *melonis*. *Journal of Plant Biochemistry and Biotechnology*. Volume 23, Issue 3, pp 238-248

Sebastiani MS and Ficcadenti N. *In vitro* plant regeneration from cotyledonary explants of *Cucumis melo* L. var. *cantalupensis* and genetic stability evaluation using RAPD analyses. (Submitted to In vitro Cellular & Developmental Biology - Plant)

Sebastiani *et al.,* Transcriptome analysis of melon-Fusarium oxysporum f. sp. melonis race 1.2 pathosystem using RNA-Seq (in preparation)

Congress Abstracts

2 - Sebastiani MS, Giardini A, Platani C, Bertone A, Ferrari V, Ficcadenti N (2014). Characterization of globe artichoke (Cynara cardunculus var. scolymus L.) spring landraces from Marche region for total phenolic compounds and antioxidant activity. 58th Annual Congress, Italian Society of Agricultural Genetics, Alghero, September 15-18, 3.17

3 - Giardini A, Sebastiani MS, Platani C, Piccinini E, Ficcadenti N, Ferrari V (2014). Effect of plantation age on health-related compounds in *Asparagus officinalis* L. 58th Annual Congress, Italian Society of Agricultural Genetics, Alghero, September 15-18, 4.12

4 - Sebastiani MS, Bagnaresi P, Sestili S, Biselli C, Orrù L, Ferrari V, De Lorenzo G, Ficcadenti N (2014). Exploring resistance response in melon-*Fusarium oxysporum* f.sp. *melonis* race 1.2 interaction using high throughput RNA sequencing. Abstract Book – Poster presentation, Plant Biology Europe FESPB/EPSO Congress, Dublin, June 22-26, p. 435

5 - Sebastiani MS, Bagnaresi P, Sestili S, Biselli C, Orrù L, Belisario A, Valè G, Cattivelli L, Ferrari V, De Lorenzo G, Ficcadenti N (2013). RNA-Seq based transcriptome analysis of melon (Cucumis melo L.) aimed at identification of candidate genes involved in the resistance towards *Fusarium oxysporum* f. sp. *melonis* race 1.2. 57th Annual Congress, Italian Society of Agricultural Genetics, Foggia, September 16-19, 7.24

6 - Finco A, Sebastiani MS, Ferrari V, Ficcadenti N (2013). An efficient regeneration protocol for genetic transformation of different melon genotypes (Cucumis melo L. var. cantalupensis). 57th Annual Congress, Italian Society of Agricultural Genetics, Foggia, September 16-19, 1.21

7 - Sebastiani MS and Ficcadenti N (2013). An efficient protocol for *in vitro* plant regeneration from cotyledon explants of different melon genotypes (Cucumis melo L. var. cantalupensis). Abstract book - Young Researches in Life Science (YRLS) Conference, Paris, May 22-24, p.129

8 - Sestili S, Sebastiani MS, Leteo F, Delvecchio S, Ficcadenti N, Campanelli G (2012). Molecular and morphological characterization of three leafy cardoon landraces (Cynara cardunculus L.) collected in Emilia Romagna Italian region and cultivated by using organic farming techniques. VIII International Symposium on Artichoke, Cardoon and their wild relatives, Viterbo, April 10-13

8. ACKNOWLEDGEMENTS

First and foremost, my thanks goes to Dr. Nadia Ficcadenti, who allowed me to carry out my work and improve my knowledge at the CRA-Research Institute for Vegetable Crops (CRA-ORA) and to my supervisor, Prof. Giulia De Lorenzo, who recommended me with her valuable technical advice.

I would also like to thank Dr Sara Sestili for her help and precious friendship.

I sincerely acknowledge Dr. Valentino Ferrari, Director of the CRA-Research Institute for Vegetable Crops (CRA-ORA), where the experimental trials discussed in this thesis were carried out, for his precious support.

I would like to express my gratitude to Prof. Patrizia Ballarini, who kindly supervised me, for giving me the chance to use the fluorescence microscope at the University of Camerino (MC).

I would like to thank my dear friend Martina for her constant support and valuable advices; I always feel close to her even though we are far away.

This work would not have been completed without the support and inspiration of my family and my beloved Giampaolo, who has always been by my side during the ups and downs of this experience. I know that he really believes in me.

I want to dedicate this thesis to my loved siblings, Lia and Marco; I hope they will always rely on me.