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Control of fruit postharvest diseases by thermotherapy: understanding the mechanisms of action by –omics approaches

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The present thesis is the result of three years of study. During my PhD I have been working in Cadriano (University of Bologna) for most of the time and 6 months at the ARS-USDA station in West Virginia. Most of the work was focused on the heat treatments application on peach ad apple fruit for the control of postharvest diseases. The last year of PhD was also dedicated to the application of the DA-meter (a portable, not-destructive device for fruit ripening stage assessment) in the shelf-life of peach fruit.

The common thread of the research was to find innovative and organic methods for reducing postharvest losses, prolonging products shelf life and maintaining acceptable quality parameters. In order to take on this challenge and considering the consumer satisfaction as the main aim, the study was conducted from different points of view.

The structure of the thesis doesn't follow the classical parameters but, in order to clearly present all the studies and results obtained, the manuscript was divided into six papers.

Except for the first chapter, all the others have the following scheme:

- Abstract
- Introduction
- Materials and methods
- Results
- Discussion
- Conclusions
- References
- Figures
- Tables

The decision to avoid a general introduction was done to have a more fluently reading of the thesis without any repetition. Moreover, in the first chapter the readers can be informed about the state of art of physical and chemical methods for the control of postharvest pathogens. The studies reported in the first three chapters are already published or *in press*.

Acknowledgments

I would like to give my thanks to all the co-authors and technicians who helped me because, without them, it would not be possible any study described. In particular my supervisors, Prof. Marta Mari and Prof. Paolo Bertolini who gave me the possibility to carry out the PhD program and also Dr. Michela Guidarelli for her professional help. I want also to thank Prof. Michael Wisniewski and his technician Erik Burchard for the special professional assistance and moral support. Last but not least Dr. Fiorella Neri and Dr Irene Cameldi, their friendship and working collaboration are one of the most important gifts received during the years of PhD.

Table of contents

Abstract

In recent years the hot water treatment (HW) represents an effective and safe approach for managing postharvest decay. This study reported the effect of an HW (60°C for 60 s and 45°C for 10 min) on brown rot and blue mould respectively. Peaches was found more thermotolerant compared to apple fruit, otherwise *Penicillium expansum* was more resistant to heat with respect to *Monilinia* spp. In semi-commercial and commercial trials, the inhibition of brown rot in naturally infected peaches was higher than 78% after 6 days at 0°C and 3 days at 20°C. Moreover, in laboratory trials a 100% disease incidence reduction was obtained by treating artificially infected peaches at 6-12 h after inoculation revealing a curative effect of HW. The expression levels of some genes were evaluated by qRT-PCR. Specifically, the cell wall genes (*β-GAL*, *PL*, *PG*, *PME*) showed a general decrease of expression level whereas *PAL*, *CHI*, *HSP70* and ROS-scavenging genes were induced in treated peaches compared to the control ones. Contrarily, HW applied on artificially infected fruit before the inoculum was found to increase brown rot susceptibility. This aspect might be due to an increase of fruit VOCs emission as revealed by PTR-ToF-MS analysis. In addition a microarray experiment was conducted to analyze molecular mechanisms underneath the apple response to heat. Our results showed a largest amount of induced *Heat shock proteins* (*HSPs*), *Heat shock cognate proteins* (*HSCs*), *Heat shock transcription factors* (*HSTFs*) genes found at 1 and 4 hours from the treatment. Those genes required for the thermotolerance process could be involved in induced resistance response. The hypothesis was confirmed by 30% of blue mold disease reduction in artificially inoculated apple after 1 and 4 hours from the treatment. In order to improve peaches quality and disease management during storage, an innovative tool was also used: Da-meter.

Keywords*:Monilinia* spp.; *Penicillium expansum*; Hot water treatment; Peach; Apple; Da-Meter.

Aims of the thesis

In the last years, the increasing demand of safer food addressed many researches to study innovative approaches for crop protection from the field to postharvest phase. Alternative methods to chemicals, such as biological control agents, natural substances and physical treatments appeared as the future of crop protection. Today the use of chemicals during the whole productive process is not already completely replaced by alternatives means however, the fungicide use was reduced and an integrated disease management might be possible .

The main aim of the present study was to clarify the mechanisms of action of a physical method such as heat, tested for the control of many fruit postharvest diseases. The research was focused on one of the "oldest organic tool" to control pathogen known in the world, the hot water (HW), applied on peaches and apples before storage. Heat treatments (thermotherapy) have been used for over a century to free plant materials from pathogens and the temperature and exposure duration determined empirically (Grondeau and Samson, 1994; Paull and Chen, 2000). Postharvest heat treatments of fruit are used for insect disinfestations, disease control, to modify fruit responses to other stresses and maintain fruit quality during storage (Paull and Chen, 2000).

The study started with the develop of a protocol of treatment in order to find the best combination of temperature and time, avoiding any physical and qualitative injuries for peaches, nectarines and apple fruit. The dipping of fruit in HW at 60 °C for 30-60 s, and 45 °C for 10 min were selected, respectively, for the application of stone and apple fruit treatment. Promising results on disease reduction obtained in laboratory and commercial trials increased our interest to study in deep the action mechanisms of the HW treatment on the selected species.

The following three chapters addressing the topic of the heat treatment, describing at first a general overview on the state of art relating to physical and chemical treatments (1). The study continued carrying out laboratory and commercial trials on peach fruit in order to assay the HW treatment against Monilinia rots on a large scale (2). Then, the molecular changes occurring in peaches metabolism after the heat treatment was investigated, focusing on the changes in expression level of cell wall genes and those involved in stress response (3).

*1.***Physical and chemical control of postharvest diseases**

Spadoni A., Neri F. and Mari M., 2014. Physical and chemical control of postharvest diseases in Advances in Postharvest Fruit and Vegetable Technology. Eds. Golding J. and Wills R., Ed. CRC Press (*in press*).

2. **Control of Monilinia rots on fruit naturally infected by hot water treatment in commercial trials**

Spadoni A., Neri F., Bertolini P. and Mari M., 2013. Control of Monilinia rots on fruit naturally infected by hot water treatment in commercial trials. Postharvest Biology and Technology 86, 280-284.

3. **Influence of hot water treatment on brown rot of peach and rapid fruit response to heat stress**

Spadoni A., Guidarelli M., Sanzani S.M., Ippolito A., and Mari M., 2014. Influence of hot water treatment on brown rot of peach and rapid fruit response to heat stress. Postharvest Biology and Technology (*in press*).

However the HW treatment applied on fruit naturally or artificially infected, showed great levels of efficacy in reducing fungal inoculum and, consequently, brown rot incidence, the results obtained on fruit heat treated and successfully inoculated with *M. fructicola* (preventive HW treatment) were not encouraging for pathogen control. For explain the ineffectiveness of a preventive HW treatment, on the possible role of peach volatile compounds (VOCs) produced by fruit immediately after HW treatment in pathogen pathogenesis was investigated (4).

*4.***Controlling Monilinia rot by heat treatment? The role of peach VOCs**

The metabolic changes after any kind of stresses might depend on two factors: the type of stress and the organism stressed. This could explain the differences in phenotypic effects and gene expression analysis found in apple fruit, compared to peaches after an HW immersion. A microarray analysis was made on "Ultima Gala" apples studying in deep the gene expression level on skin heated for seven time points within 24 hours. The experiment was carried out to the Appalachian Fruit Research Station, USDA-ARS, located in Kearneysville (West Virginia, USA) (5).

*5.***Transcriptional profiling of apple fruits in response to heat treatment: involvement of a defense response during *P. expansum* infection**

Stress derived from a postharvest heat treatment leads to an alteration of gene expression and fruit ripening that can sometimes be either delayed or disrupted. The extent of the alternation of fruit ripening is a function of the exposure temperature, duration and quick cool of fruit following the heat treatment.

In order to improve peaches quality and disease management during post-harvest phase, an innovative technique was tested. A new portable device (Da-Meter) introduced to monitor peach ripening from field to starage was applied at harvest to determine the best commercial chain of peach fruit correlating to brown rot susceptibility (6).

6. **The DA meter, a dynamic tool for rapid evaluation of peach shelf-life: a preliminary study**

Physical and chemical control of postharvest diseases

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1.0 Introduction

Fruit and vegetables are highly perishable produce and after harvest, are subjected to considerable quantitative losses. Since they are rich in water and nutrients, are an ideal substrate during storage for the development of pathogenic microorganisms such as *Penicillium* spp., *Monilinia* spp., *Botrytis cinerea*, etc. Waste during the postharvest phase also represents an important economic loss, which is greater the closer it is to the sale of the produce, considering the added value of fruit after harvest, storage, transport and commercialization. The extent of postharvest losses varies in relation to commodities and country and although few up-to date data are available, losses can be estimated as ranging from a minimum of 10-15% in countries with advanced technologies, to over 50% in developing countries (Wilson and Wisniewski, 1989). In addition, qualitative losses such as loss in edibility, nutritional quality, caloric value, and consumer acceptability of the products are much more difficult to assess than quantitative losses (Kader, 2005). Moreover, some postharvest pathogenic fungi such as *Penicillium* spp., *Aspergillus* spp. etc. represent a serious concern to human health since they are producers of several toxic compounds (e.g. patulin, citrinin, chaetoglobosins, ochratoxin A, etc.) that can affect the safety of both fresh fruit and fruit-based products (Andersen et al., 2004; Guzev et al., 2008). The natural resistance of fruit to disease declines with ripeness and storage duration, although the use of appropriate postharvest technologies (controlled and modified atmosphere, ultra-low oxygen levels, etc.) has greatly reduced losses from production to retail in more developed countries (Kader, 2005). Fungicide treatments still remain one of the most effective methods to reduce postharvest decay, since they protect the fruit from infections occurring before treatment, including quiescent infections, as well from infections during storage, handling and marketing (Adaskaveg and Forster. 2009). However, the repeated and continuous use of fungicides has led to a strong selection pressure in pathogen populations, resulting in the development of resistance to some common fungicides like benzimidazole, demethylation inhibitors, dicarbossimide, etc. Moreover, in the last 25 years, the concern about people's health and the environment determined by chemical treatments has intensified consumer demand of fruit with low fungicide residues, sustainable agriculture, integrated crop management and organic production (Directive 2009/128/EC). Multiple retailers have identified the occurrence of pesticide residues as one of the prime concerns of consumers regarding fresh produce, leading them to pursue a policy of fruit residue reduction to 3-4 active ingredients or to their

complete elimination (Cross and Berrie, 2008). In this context, considerable efforts are devoted by researchers to finding safer methods for fruit disease control. This review deals with the substantial progress achieved in the use of physical and chemical control measures, also taking into account constraints and obstacles that still make their widespread diffusion and practical application difficult.

1.1 Physical control of postharvest diseases

1.1.1. Heat treatments

Heat treatments can be applied in the form of hot water, vapour heat, hot air or hot water rinse brushing. While they were originally used for insect disinfestation (Lurie, 1998) and disease control, the application of heat in various forms has also been utilized to prevent the development of disorders triggered by cold storage like chilling injury. Hot water treatments were first reported in 1922 to control decay on citrus fruit (Fawcett, 1922); extensive reviews have subsequently dealt with the possibility of using heat to prolong the shelf life of fruit and vegetables and to control quarantine insects (Fallik, 2004). Among various non-chemical approaches, hot water treatment appears to be one of the most effective and promising methods, especially useful for organic crops, to control relatively high rates of postharvest decay in environmentally friendly ways (Mari et al., 2007; Liu et al, 2012, Li et al, 2013). Hot water dipping (HWD) is a heat treatment applied by complete immersion of vegetables and fruits in warmed water (above 40 °C). Many fruits and vegetables can tolerate temperatures of 50-60°C for 5-10 min without quality losses and phytotoxicity appearance (Barkai-Golan and Phillips, 1991; Lurie S., 1998; Janisiewicz and Conway, 2010) but the duration of the treatment must be carefully controlled to avoid heat damage (Vigneault et al, 2011). Treating plums by immersion in pre-warmed water at 60°C for 60 s was effective against brown rot, reducing the incidence of disease by more than 95% with respect to the control (Karabulut et al., 2010). The activity of HWD depends on at least two components; the first is a direct and lethal action of heat on fungal inoculum as spores and mycelium present on the fruit surface or in the first layers of peel. The second component could be an indirect action of heat on the host, mediated by a stress-induced response of fruit (Maxin et al., 2012), including disease resistance. The efficacy on fungal eradication depends on genetic differences among fungi. *M. fructicola*, for instance, is more heat sensitive than *P. expansum* (Barkai-Golan and Phillips, 1991) but more resistant than *M. laxa* and *M. fructigena* (Spadoni et al., 2013). Regarding the host response, several studies have indicated that heat treatment could induce a stress response of fruit (Liu et al., 2012): however, more investigations are required to better elucidate the molecular mechanisms involved. In addition, it has been reported that appropriate heat treatment delayed softening and extended postharvest life of fruit such as nectarine (Fruk et al., 2012), peach (Bustamante et al.,

2012), papaya (Li et al, 2013), banana (Promyou et al., 2008), and citrus (Yun et al., 2013). It is also effective for delaying ripening in several fruit species (Paull, 1990; Lurie, 1998), contributing to reducing fruit softening (Martinez and Civello, 2008) and better preparing peach fruit for a subsequent storage (Lauxmann et al., 2012), avoiding or reducing the effect of some abiotic or biotic stress on fruit quality. At molecular level, the main factor involved seems to be the expression of heat shock genes encoding different heat shock proteins (HSPs). It is known that HSPs perform a critical function in refolding partially denaturated protein, completing degradation of denaturated proteins, assisting the *ex novo* protein synthesis and averting protein aggregation (Aghdam et al., 2013). HWD might be a good solution to improve fruit postharvest life but some negative aspects have to be considered. As reported by Schirra and D'hallewin (1997), prestorage dipping of 'Fortune' mandarins in water at 50-54 °C significantly inhibited chilling injury but increased the fruit weight loss. Moreover, while this method has the potential to reduce the natural inoculums present on the fruit surface, it is not always compatible with fresh-cut plant foods since it may reduce consumer acceptability due to undesirable changes of flavour, texture, colour and nutritional quality (Maghoumi et al., 2013). Although HWD requires specialized equipment, it might result in substantial economic savings since in the industrial packinghouse the heating of the water can be obtained by refrigerant gas (ammonia, hydrochlorofluorocarbon, *etc*.) coming from the cooling plant of the stores; indeed, the heat of this gas is usually dispersed in the environment through the condensers.

A more recent technology than HWD is hot water rinsing and brushing (HWRB). HWRB treatments are extensively studied because they require a higher temperature and a shorter exposure time than traditional hot water dips. HWRB treatments could not only remove the heavy dirt, pesticides and fungal spores on freshly harvested produce, but they could also improve general product appearance and maintain product quality (Fallik, 2004). The method involves the fruit rolling over brushes directly into the pressurized recycled hot water rinse at temperatures between 48°C and 63°C for 10–25 s (Fallik, 2004). HWRB treatments possess the ability to remove fungal pathogens from the fruit surface as a result of the brushing effect, while the natural wax platelets could be melted and smoothed to seal the stomata openings on the fruit surface (Fallik 2004). It has been demonstrated that the use of HWRB at 55°C for 12 s for sweet peppers is a practical strategy for reducing weight loss, chilling injury and softening and preserving nutritional quality, especially the antioxidant activity (Ilić et al., 2012). This methodology, applied for apple fruits, did not have such good results as HWD. In fact, the main advantage of hot water immersion is a uniformly consistent temperature profile throughout the treatment tank at or slightly above the set point temperature, while in the case of HWRB a possible non uniform coverage of fruit by hot water could result in limited pathogen control.

However, HWRB has the potential to become a sustainable alternative for fruit disease control because it is less costly than HWD and because its short treatment times enable it to be integrated into existing fruit grading (Maxin et al, 2012).

Hot air treatment, also called 'curing', consists of holding the fruit at 30 to 37°C and at high relative humidity (90-98%) for 65 to 72 h. The treatment has been used for the control of both fungal decay and insects. Forced hot air is preferred by many countries for the development of quarantine procedures (Vigneault et al, 2013). However, the method based on curing treatments has also been evaluated to control green and blue moulds on citrus fruit (Zhang and Swingle, 2005; Montesinos-Herrero et al., 2011) proving an effective alternative to fungicides. In apple fruit, hot air treatment at 38°C for 4 days has been considered the optimum to preserve postharvest storage quality (Klein and Lurie, 1991). It can delay ripening and maintain firmness of apple fruit to improve consumer acceptability, also controlling the development of common postharvest diseases caused by *P. expansum*, *B. cinerea* and *C. acutatum* (Klein et al. 1997; Conway et al. 2005; Shao et al. 2007). As recently reported by Zhang et al. (2013), the application of curing on tomato fruit (38°C for 12 h) activated arginine catabolism and, consequently, reduced the susceptibility to chilling injury. In addition, forced hot air treatment for prolonging postharvest shelf-life of mango did not cause injury in fruit but the moisture content of the heating air differentially modulated the postharvest ripening; in particular, moist air temporarily slowed down the ripening process of mangoes (Ornelas-Paz and Yahia, 2013). Curing can also induce increased residual activity (Shao et al., 2009) and enhance the wound healing process (Shao et al., 2010), controlling fungal diseases in 'Fuji' apple fruit. However, some negative impacts including enhanced yellowing of peel, reduced titratable acidity and weight were observed after heat treatment in apples. These three factors are regarded as the signals of senescence for fruit. In a previous study, Lurie and Klein (1992) suggested that these adverse outcomes of heat treatment can be reduced when combined with a calcium dip.

A specific curing application to kiwifruits against stem-end rot caused by *B. cinerea* is considered an important tool in view of a reduction in fungicide treatment. In this case, curing lies in keeping fruits at ambient temperature for at least 2 days before cold storage (Mari et al., 2009). During the early phases of wound healing, an active phenol metabolism was observed in the fruits and it probably provided the monomers required for the suberisation of picking wound, the main infection site of *B. cinerea* in kiwifruits. The conspicuous increase in phenylalanine ammonia-lyase and polyphenolxidase activity shown by cured fruits respectively indicated the activation of a resistance mechanism and an oxidative phenolic polymeralisation which further increased tissue resistance to the pathogen (Ippolito et al., 1995). Currently, for

grey mould management in kiwifruits, curing is carried out directly in cold storage rooms, decreasing the temperature from 10 to 1°C in 8 days and delaying establishment of the controlled atmosphere regimes to 30-40 days after harvesting. This method reduced the incidence of stem end rot and at same time, no negative effects were observed on fruit quality (Brigati et al., 2003).

Vapour heat is a method of heating commodities that uses air saturated with water vapour at temperatures of 40 to 50°Cto kill insect eggs and larvae as a quarantine treatment before fresh market shipments (Vigneault et al, 2011). Heat treatment is by condensation of hot water vapour on the cooler fruit surface. This procedure was first used to kill Mediterranean and Mexican fruit fly (Janick, 2008). The method is disputed because the temperature of the fruit is even higher than that of the surrounding water vapour media during treatment (Vigneault et al, 2011; Shellie and Mangan, 2000), sometimes causing damage to the fruit undergoing treatment (Vigneault et al, 2011). Moreover, the treatment could be difficult to apply and other studies are required to better understand the steam distribution on the fruit surface.

Heat based treatment has a greater advantage than other non-conventional control methods of fruit disease such as natural compounds and GRAS, since it does not require any registration from Public Agencies and in addition HWD or curing appear particularly attractive to the fruit industry since it could be immediately utilized and incorporated into handling practices before storage without extensive technical modifications.

1.1.2 Irradiation

The process involves exposing food to a controlled amount of ionizing radiations for a specified period. It is similar to ultraviolet (UV), visible and infra-red light, microwaves and radio waves used for communication. The effects of irradiation on the food have been studied extensively for a long time (Burton et al., 1959) and well reviewed by Arvanitoyannis et al. (2009). Only in the last decades, due to the strong desire to reduce the chemical fungicides applied to fruits and vegetables, the investigations on the use of irradiation are increased exponentially, and irradiation technology has proved to be effective in reducing post-harvest losses, controlling the stored product against insects and microorganisms. Internationally, food irradiation has been considered a safe and effective technology by the World Health Organization (WHO), the Food & Agriculture Organization (FAO), and the International Atomic Energy Agency in Vienna (El-Samahy et al., 2000). Application of UV-C controlled *P. expansum* of apples (Capdeville et al., 2002), postharvest rot of strawberries (Marquenie et al., 2002) and Rhizopus soft rot of tomatoes (Stevens et al., 1998). The activity of UV-C, assayed in *in vitro* trials, showed an inhibition of mycelial growth of *Monilinia* spp. after long-wave (320-380nm) UV exposure (De Cal and Melgarejo, 1999), but no effect against brown rot after

UV-C light treatment was observed in cherries (Marquenie et al., 2002) and in peaches (Bassetto et al., 2007). In addition, radio frequency energy was proposed not only to heat foodsbut also to disinfect commodities and to control stone fruit postharvest diseases such as brown rot (Casals et al. 2010). The effect of UV radiation has been associated with both germicide properties as well as physiological response like activation of defence mechanisms. Charles et al. (2009) found changes in the protein content and profile of tomato fruit treated with UV-C (3.7 kJ m^{-2}) at mature green stage. In particular, the results showed the synthesis repression of two proteins detected in senescing control fruit and the induction of stress- and/or pathogenesis-related proteins: both aspects could be involved in the reduction of *B. cinerea* incidence. Moreover, UV-C can affect quality attributes in relation to species and doses: treated papaya maintained a higher firmness than the control (Zhao et al., 1996), while strawberry and apple firmness decreased as the irradiation dose increased (Yu et al., 1996; Drake et al., 1999). Sensory qualities of grapefruit after exposure to gamma irradiation (0.4kGy) were comparable to the control after 35 days storage (Patil et al., 2004); similarly, irradiated melon (2.5kGy) maintained quality within acceptable limits (Bibi et al., 2006). Pineapple fruit after treatment with gamma irradiation (0.15 kGy) showed better texture than the control (Susheela et al., 1997), although the appearance of ten citrus cultivars was negatively affected by the loss of glossiness after treatment with 0.45kGy dose (Miller et al., 2000).

1.2 Chemical control of postharvest diseases

1.2.1 General Regarded as Safe

Chemical products with low toxicity as generally recognized as safe (GRAS) compounds have received an increasing interest for the control of postharvest fruit disease. GRAS is a US Food and Drug Administration designation and denotes a substance added to food that is considered safe by experts and for this reason is exempt from the usual Federal Food, Drug, and Cosmetic Act food additive tolerance requirements (Senti, 1981). GRAS are allowed with very few restrictions for many industrial and agricultural applications by regulations worldwide and offer a considerable promise in postharvest technology, showing antimicrobial, antifungal, and insecticidal properties (Gregori et al., 2008; Fagundes et al., 2013). Peracetic acid, K-sorb, sodium bicarbonate, calcium salts are only some examples of GRAS widely used in the food industry for leavening, pH control, taste and texture development. They demonstrate a broad-spectrum of antimicrobial activity, inhibiting the development of postharvest diseases such as blue mould apple decay (Janisiewicz et al., 2008), *M. laxa* and *Rhizopus stolonifer* of stone fruits (Mari et al., 2004) and green mould and sour rot of citrus (Smilanick et al., 2008). In spite of theinteresting results obtained in laboratory and small scale experiments,salt usage limitations, as inconsistent activity and limitedpersistence, lack of preventive effect, risk of fruit

injury, make their postharvest commercialapplication still unreliable (Larrigaudiere et al., 2002; Palouet al., 2002). However, pre-harvest salt treatments (a few days before harvest) seem to overcome these issues, completely inhibiting the incidence of decay in citrus fruits (Youssef et al., 2012) or showing an efficacy higher than or similar to that of conventional chemical treatments against storage rots of table grapes (Nigro et al., 2006). The antifungal activity of salts against several pathogens is correlated to a direct inhibition of spore germination, germ tube elongation and production of pectinolytic enzymes (Hervieux et al, 2002). However, indirect effects must also be taken into consideration, such as a possible increase of tissue resistance with structural changes in cell wall or phytoalexin production induced for example by sodium carbonate (Venditti et al., 2005). In addition, the osmotic stress caused by the presence of salts in field applications could contribute to a decrease in epiphytic fungal populations included *Pencillium* spp. species (Youssef et al., 2012).

Other chemicals such as chlorine dioxide, hydrogen peroxide, citric acid, and ethanol are listed as GRAS substances, and their application in postharvest phase by fogging could be very useful, since some fruits like strawberries require handling and wetting to be minimized. Vardar et al. (2012) obtained a significant reduction of postharvest decay in strawberries treated by fogging with hydrogen peroxide (2000 μLL^{-1}) such as in microbial populations in treated fruit surface and in the storage atmosphere. In addition, fruit and vegetables are considered the major vehicles for transmission of food-borne enteric viruses since they are easily contaminated at pre- and postharveststages and the sanitizers commonly used are relatively ineffective for removing human norovirus surrogates from fresh produce. Some GRAS, such as polysorbates, were able to achieve a 3-log reduction in virus titre in strawberries and an approximately 2-log reduction in virus titre in lettuce, cabbage, and raspberries (Predmore and Li, 2011).

In 1997, ozone was declared to be a GRAS for food contact applications (EPRI Expert Panel, 1997) and since then ozone-based treatment of fresh fruit and vegetables has been used in the postharvest handling industry, achieving satisfactory results. In a preliminary report, the inhibition of conidia of *B. cinerea*, *M. fructicola*, *P. digitatum,* and *R. stolonifer* required a concentration of more than 200 μLL^{-1} under humid conditions and 4000 μLL^{-1} under dry conditions (Margosan and Smilanick, 1998). Unfortunately, the concentrations of ozone that inactivated conidiawere relatively high and cannot be used without complete containmentof the gas and protection of workers from it. Under conditionswhere ozone is present during an 8 h workday, gas concentrations cannotexceed 0.075 $\mu L\ L^{-1}$ (USEPA, 2008). Ozone was extensively tested for the control of table grape decay (Smilanick et al., 2010). Although it is fungistatic, dose dependent, and at high concentrations (above 5000 $\mu L\ L^{-1}$) can be phytotoxic, a treatment with 5000 $\mu L\ L^{-1}$of ozone in a commercial chamber reduced gray mould incidence

from natural inoculum by about 50% after 6 weeks storage at 0°C (Gabler et al., 2010). Similarly, kiwifruit continuously exposed for 4 months to gaseous ozone (0.3 μLL^{-1}) showed a delayed development of stem-end rot and a 56% reduction of grey mould incidence. The observed disease suppression strongly suggests that ozone treatments induce resistance of kiwifruit to the pathogen. Measurements of antioxidant substances and antioxidant activity on fruit exposed to ozone for the same time intervals showed a strong negative correlation between disease incidence or severity and phenol content (Minas et al., 2010).

1.2.2 Electrolyzed water

Electrolyzed water (EW) is generated by the electrolysis of salt solution through an electrolytic cell where the anode and cathode are separated by nonselective membranes. EW was initially developed in Japan as a medical product and it was successfully applied as a noble sanitizer to inactivate microorganisms on food and food processing equipment surfaces (Al-Haq et al., 2002). Several studies have also documented the strong antifungal activity of EW against postharvest diseases such as brown rot of peaches (Guentzel et al., 2010), green mould of tangerine (Whangchal et al., 2010) and blue mould of apple (Okull and Laborde, 2004). In all of these investigations, electrolysis was conducted with the addition of sodium chloride as the electrolyte, with consequent formation of free-chlorine and chlorinated organic compounds like chloramines, dichloramines and trichloromethanes, inducing drawbacks for handlers and consumers. Moreover, free chlorine is quickly inactivated by the heavy inorganic load present in the wash water of commercial packinghouses; therefore, the use in the electrolysis reaction of salts not containing chlorine might be particular interesting (Fallanaj et al., 2013). Compared to other conventional methods of disinfection, EW reduces the treatment time, is easy to obtain, has very few side effects, and is relatively cheap (Tanaka et al., 1999). In addition, it did not negatively affect the organoleptic properties, colour, scent, flavour, or texture of the various food commodities (Al-Haq et al., 2005). However, the main advantage of electrolyzed water is its safety for humans and the environment; when it comes into contact with organic matter, or is diluted with tap water or water produced by reverse osmosis, it reverts to normal tap water (Huang et al., 2008). In 2002, Japan officially approved EW as a food additive (Yoshida et al., 2004). The main mechanism of action of EW relates to the oxidation that could damage cell membranes, create disruption in cell metabolic processes and essentially kill the cell. Spore treatment with EW involves cell structural changes as well reactive oxygen species (ROS) accumulation, mitochondrial membrane integrity, and ATP production. A significant increase of ROS accumulation was observed in *P. digitatum* spores exposed to the electrolyzed salt solution for 15 min. However, other factors can be involved in the production of ROS such as

pH increase; in fact, the pH gradient was in the range 8.5-9.0 after the electrolysis process (Fallanaj, 2012).

1.3 Natural compounds

1.3.1 Volatile organic compounds from plants

Plants produce an amazing diversity of secondary metabolites (more than 100,000 have been identified and at least 1,700 are volatile) having a wide range of biological activities (Boulogne et al., 2012; Bitas et al., 2013). Some secondary metabolites, occurring in health plants in their active forms or as inactive precursors, are associated with the defence system and have shown potential for the control of postharvest diseases. Most of them proved to directly inhibit pathogens by harmful effects on fungal membranes (Fallik et al., 1998; Arroyo et al., 2007), but some, such as flavonoids, enhanced the defence responses of the host (Sanzani et al., 2010). Plant volatile organic compounds (VOCs) are substances with low molecular weight and high vapour pressure that are naturally emitted by different organs (i.e. leaves, buds, flowers, fruits, bark, wood, roots). Their high volatility at ambient temperature makes them suitable for postharvest biofumigation, a technique for disease control that offers the advantage of minimal handling and absence of fruit wetting. Studies carried out in the last 20 years have produced significant progress in our knowledge on the antifungal activity of plant metabolites, and more than 20 volatile compounds from edible plants (spices, herbs, fruits, vegetables) were found to be particularly interesting as novel means for decay control because of their safety at low concentrations.Most of these compounds are also widely used as food additives, and the Joint FAO/WHO Expert Committee on Food Additives expressed no safety concerns at current levels of intake for allyl-isothiocyanate (AITC), *p*-anisaldehyde, carvacrol, (-) carvone, *trans*-cinnamaldehyde, hexanal, *trans*-2-hexenal, 2-nonanone, terpineol and thymol, when used as flavouring agents. Although different forms of application (liquid or vapour phase) and measurements of pathogen inhibition (mycelial growth and/or conidial germination) applied in the studies often it makes difficult to compare the minimal inhibitory concentrations (MICs) obtained. The most consistent fungicidal activity by plant bioactive compounds was found with some isothiocyanates (ITCs), followed by *trans*-2-hexenal, *trans*-2-nonenal, carvacrol, thymol, citral and *trans*-cinnamaldehyde and reviewed by Mari et al. (2011). The main factors involved in the antimicrobial activity of the compounds proved to be functional groups, hydrophobicity and vapour pressure (Andersen et al., 1994; Caccioni et al., 1997; Arfa et al., 2006). The *in vitro* inhibition by plant compounds has not always been confirmed in *in vivo* assays. Besides chemical characteristics, other factors proved to influence the effectiveness of antifungal compounds in disease control, including treatment conditions (form of application, concentration, temperature, exposure time, time of treatment, formulation) and characteristics

of the pathogen (age and form of infection structures, location of pathogen in host tissue). Different levels of sensitivity to treatments were found among fruit species or cultivars and detrimental effects on sensory traits (odour, texture and flavour) or phytotoxic symptoms on fruit have been observed in some studies with treatments effective in disease control (Vaughn et al., 1993; Neri et al., 2006 c, 2007; Mehra et al., 2013).

Isothiocyanates (ITCs) derive mainly from hydrolysis of *Brassica carinata* defatted meal containing glucosinolates. The ITCs showed a strong activity against a wide range of food pathogens in specific biological tests (Delaquis and Mazza, 1995). Some ITCs are volatile substances and could potentially be successfully employed in treatments as biofumigation. The postharvest phase, characterized by restricted environment parameters such as temperature, relative humidity and atmosphere gas composition, represents an advantage for biofumigation of fresh fruit before storage. In *in vitro* tests, AITC, a volatile ITC, showed significant inhibition of conidia germination and/or mycelial growth of *M. laxa*, *P. expansum* (Mari et al., 1993), *Fusarium oxysporum* (Ramos-Garcia et al., 2012), *B. cinerea* (Ugolini et al., 2014), while benzyl ITC inhibited *Alternaria alternata* mycelial growth, a postharvest tomato pathogen (Troncoso-Rojas et al., 2005). In *in vivo* trials, ITCs were found to be active against numerous postharvest pathogens and on different hosts. However, their activity did not always confirm the results obtained in preliminary *in vitro* tests, showing that the treatment conditions should often be established not only in relation to the active substance and fungal pathogen, but also to fruit and vegetables' response to treatment. Since many ITCs have been synthetized, few data reported any activity of ITCs produced *in situ*, although their effectiveness was similar; in fact, synthetic and glucosinolate-derived AITC vapourswere evaluated against *B. cinerea* on strawberries and no significant differences were found between two origins (Ugolini et al., 2014). This is an important aspect, showing that biofumigation could be used for industrial applications; the use of bio-based chemicals obtained from renewable natural resources also fits well with the goals of a sustainable agriculture. The mechanism by which ITCs inhibit fungal growth is not yet completely known. Probably, a non-specific and irreversible interaction of the ITC with the sulfidryl groups, disulphide bonds and amino groups of proteins and amino acids residues can take place (Banks et al., 1986). Despite numerous data on antifungal, antibacterial, anti-nematode and anti-insect activities of ITCs, only a few investigations reported their effects on treated fruit quality and residue content. The postharvest quality of bell peppers represented by general appearance (absence of phytotoxic symptoms), weight losses, firmness, titrable acidity and total soluble solids was not affected by the mixture of ITC treatment (Troncoso-Rojas et al., 2005). Similar results were obtained in strawberries, where total phenolic content and antioxidant capacity estimated in treated and untreated fruits showed no significant

difference between them. In addition, residue analysis performed on fruit at the end of storage (7 d after treatment) showed very low values (< 1 mg kg^{-1}) (Ugolini et al., 2014).

***trans*-2-Hexenal**is C_6 an α,β-unsaturated aldehyde naturally occurring in olive oil, tea and most fruits and vegetables. The compound is also used as a flavouring agent and its estimated daily intake in Europe and the USA (791 and 409 μg/person per day, respectively) is below the threshold of concern (1800 μg/person per day), showing no safety concerns at current levels of intake. The compound is known for its antimicrobial properties and is thought to be involved in the defence mechanism of plants. Its production, together with other C_6 aldehydes and alcohols named "green leafy volatiles", increases rapidly in damaged plant tissues, as a result of the activation of the lipoxygenase hydroperoxide lyase enzymatic pathway in response to wounding and herbivore or pathogen attack (Matsui, 2006). *trans*-2-Hexenal was shown to have strong antimicrobial activity against many postharvest pathogens (*B. cinerea, C. acutatum, Helminthosporium solani, M. laxa, Pectobacterium atrosepticum, P. expansum*) *in vitro* and *in vivo* tests and its inhibition has been found particularly marked against the fungal conidial form (Vaughn et al., 1993; Fallik et al., 1998; Neri et al., 2006 a, 2007; Arroyo et al., 2007; Wood et al., 2013). The high electrophilic properties of the carbonyl group adjacent to the double bond make *trans*-2-hexenal particularly reactive with nucleophiles, such as protein sulphydryl and amino groups of the pathogens. In a study where *B. cinerea* was exposed to a radiolabelled mixture of *cis*-2-hexenal and *trans*-2-hexenal, it was demonstrated that fungal proteins, and particularly proteins of the surface, were targets of the C_6 aldehydes (Myung et al., 2007). C_6 aldehydes were preferentially incorporated into conidia rather than mycelia, a result correlated to the greater sensitivity of spore germination than mycelial growth to *trans*-2-hexenal. Postharvest exposure of pome fruits to *trans*-2-hexenal vapour, even for short times (2-4 hours at 20°C), was found to significantly reduce the infections of *P. expansum*, while 8 hours treatment was needed to significantly reduce patulin content in 'Conference' pears (Neri et al., 2006a,b). Treatment with *trans*-2-hexenal showed a curative activity up to 72 hours in blue mould control and cold storage temperature after exposure of fruit to *trans*-2-hexenal tested in 'Conference' pears did not affect the activity of the compound (Neri et al., 2006b). The timing of treatment was particularly important. Fumigation with *trans*-2-hexenal applied immediately after inoculation (2 hours) was effective against *M. laxa* and *B. cinerea*, but was generally not useful to control *P. expansum.* In 'Golden Delicious' apples, *P. expansum* and patulin content were greatly reduced by the *trans*-2-hexenal treatment (12.5 μL L^{-1}) applied 24 hours after inoculation, without negative effects on quality traits (Neri et al., 2006c). In contrast, concentrations effective in decay control caused phytotoxic effects in apricots, peaches, nectarines, 'Abate Fetel' pears and strawberries, and they affected fruit flavour in plums,

strawberries, 'Conference' and 'Bartlett' pears and 'Royal Gala' apples (Neri et al., 2006b,c, 2007).

The corresponding saturated aldehyde **hexanal** was shown to inhibit the growth of several postharvest pathogens *in vitro* but at concentrations higher than *trans*-2-hexenal (Caccioni et al., 1995; Neri et al., 2006 a, 2007; 2009 a; Arroyo et al., 2007; Song et al., 2007). Fumigation with hexanal (900 μL L^{-1} for 24 hours at 20°C) significantly reduced the incidence of decayed fruit in raspberry and blueberry and lesion development in peaches artificially inoculated with *M. fructicola* (Song et al., 2007, 2008). Although 40 μmol/ L^{-1} of hexanal was effective for killing the majority of *P. expansum* spores, 'Golden Delicious' apples exposed to this concentration of hexanal for 48 hours showed symptoms of phytotoxicity (Fan et al., 2006). Continued exposure to hexanal (40-70 μL L^{-1} for 7 days) effectively suppressed grey mould in tomato; however, the tomato respiration rate increased about 50% and reddening was slowed (Utto et al., 2008).

Carvacrol, a monoterpenoid phenol with a warm and pungent odour, is the character-impact constituent of oregano essential oils (*Origanum vulgare,O. onites, Thymus capitatus),* in which it occurs at concentrations of 60-80%.It exhibited fungicidal activity against a wide range of postharvest pathogens (*B. cinerea, G. candidum, M. laxa, N. alba, P. expansum, R. stolonifer*) and in particular a consistent inhibition of mycelial growth (Plotto et al., 2003; Neri et al., 2006 a, 2007, 2009). Comparable fungicidal activity was exhibited by the carvacrol isomer **thymol** (Plotto et al., 2003). This compound has a strongly aromatic, burnt, medicinal odour and occurs mainly in thyme. The antimicrobial activity of carvacrol and thymol has been ascribed to the hydrophobicity of the compounds, the presence of a phenolic hydroxyl group in these molecules and an adequate system of delocalized electrons (double bonds) that allow the OH group to release its proton (Arfa et al., 2006). The chemical structure of these molecules would allow these compounds to act as proton exchangers, reducing the gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death. Postharvest fumigations with carvacrol or thymol were effective in controlling *B. cinerea* and *M. fructicola* in cherries (Tsao and Zhou, 2000) and *M. fructicola* on apricots and plums (Liu, et al., 2002).However, these treatments caused phytotoxic symptoms and off-flavours in cherries and a firmer texture and phytotoxicity in apricots. Phytotoxicity after carvacrol or thymol exposure was also observed in oranges (Arras and Usai, 2001) and tomatoes (Plotto et al., 2003). Addition of a mixture of carvacrol, thymol and eugenol inside active packaging was effective in reducing decay in table grapes (Guillen et al., 2007), but, as confirmed also in our unpublished trials on cv Italia, fumigation with these compounds caused off-flavours in grapes. Exposure to carvacrol vapours failed to control blue

mould in pears and only slightly controlled brown rot in peaches and lenticel rot in apples (Neri *et al.*, 2006a, 2007, 2009). **Citral,** naturally occurring with the isomers neral and geranial, is an acyclic α,β-unsaturated aldehyde mainly contained inside oil glands of lemon and lime peel and in essential oils of many plants. The acceptable daily intake established for citral by the Joint FAO/WHO Expert Committee on Food Additives (2003) is ≤ 0.5 mg/Kg body weight; the compound is considered a GRAS in the United States. Nevertheless, the antifungal activity of citral against several postharvest pathogens has been well documented by *in vitro* trials (Wuryatmo et al., 2003; Palhano et al., 2004; Neri et al., 2006 a, 2007, 2009; Zhou et al., 2014), postharvest fumigation of fruit with citral showed a low degree of efficacy in blue mould and brown rot control (Neri et al., 2006a and 2007) and failed to control lenticel rot (Neri, 2009a). Treatment with citral caused severe phytotoxicity on tomato fruits, either when evaluated as a pure compound or as the main constituent of lemongrass essential oil (Plotto et al., 2003). As for *trans*-2-hexanal, the fungicidal activity of citral has been ascribed to the high electrophilic properties of the carbonyl group adjacent to the double bond. ***trans*-Cinnamaldehyde,** an aromatic aldehyde with the typical odour of cinnamon, is the main constituent of essential oils of cinnamon bark and cassia bark and leaves. Its fungicidal activity against postharvest pathogens (*Botryodiplodia theobromae, C. gloeosporioides, C. musae, Glicephalotrichum microchlamydosporum, M. laxa, N. alba, P. expansum, P. digitatum, R. stolonifer*)has been demonstrated in *in vitro* studies (Sivakumar et al., 2002; Utama et al., 2002; Neri et al., 2006a, 2007, 2009). Exposure of rambutan to *trans*-cinnamaldehyde vapours significantly reduced fungal infections without any negative effect on fruit, while treatment in an aqueous solution of *trans*-cinnamaldehyde caused phytotoxic symptoms (Sivakumar et al., 2002). Vapour treatment with *trans*-cinnamaldehyde failed to control blue mould in pears, brown rot in peaches and lenticel rot in apples (Neri et al., 2006a, 2007, 2009).

1.3.2 Essential oils

Essential oils are extracts, generally obtained by steam distillation or cold-pressing methods, from various organs of aromatic plants, and contain a complex mixture of compounds (up to 100) with diverse chemical structures, many of which are volatile. Some essential oils have demonstrated an inhibitory activity on postharvest pathogens, although at concentrations usually much higher than single plant bioactive compounds. Most essential oils are characterized by one-three main components which impart the characteristic odour or flavour to the oil and are generally the bioactive ingredients; other oils have a large number of major constituents. The achievement of essential oils with constant composition could be a critical aspect for their practical use, since quantity and quality of components of essential oils can vary depending on climate, soil composition, harvest period, chemical polymorphism in populations

and method of extraction, and this may influence the antimicrobial properties of essential oils. Instead, the possible synergism in antimicrobial activity among different components, also occurring as minor molecules, could be one of main advantages of the use of essential oils. In addition, the mixture of a variety of functional groups could reduce the risk of resistance development by pathogens. In studies comparing the antifungal activity of several essential oils, those containing mainly carvacrol (*T. capitatus, O. compactum*) or thymol (*T. glandolosus, T. vulgaris, O. vulgare* and *Syzygium aromaticum*) have been shown to exhibit the highest inhibition of many postharvest pathogens (*A. citri, B. cinerea, Geotrichum candidum, P. digitatum, P. italicum, R. stolonifer*) (Arras and Usai, 2001; Bouchra et al., 2003; Daferera et al., 2000; Plotto et al., 2003; Barrera-Necha et al., 2008). Essential oils rich in citral also showed a broad spectrum of antifungal activity (Shahi et al., 2003; Palhano et al., 2004; Lazar-Baker et al., 2011). Different results with the application of essential oils have been found in *in vivo* assays. Exposure to vapours of thyme oil caused severe phytotoxic symptoms on peaches (Arrebola et al., 2010). Among essential oils rich in citral, lemongrass (*Cymbopogoncitratus*) completely controlled the development of *P. expansum* and *B. cinerea* infections on apples (Shahi et al., 2003), while it showed a low reduction of *B. cinerea, P. expansum* and *R. stolonifer* infections in peaches (Arrebola et al., 2010). In addition, the application of lemon myrtle (*Backhousia citriodora*) essential oil (250 µl L^{-1}) reduced the incidence of *M. fructicola* rot only on non-inoculated nectarines (Lazar-Baker et al., 2011). Among other essential oils, spray application of laurel oil (3 mg mL^{-1}), containing several components (mainly 1,8-cineole, linalool, terpineol acetate and methyl eugenol), showed good antifungal activity against *M. laxa* in peaches, while it exhibited less control of *B. cinerea* in kiwifruits and *P. digitatum* in oranges and lemons (De Corato et al., 2010). Spray emulsion of cinnamon (*Cinnamomum zeylanicum*) essential oil on bananas showed better control of crown rot than clove (*S. aromaticum*) essential oil (0.2%), while it failed to control anthracnose disease (Ranasinghe *et al.*, 2005). *Vice versa*, dip treatment with clove essential oil (50 µg L^{-1}) showed a higher efficacy in reducing natural infections than cinnamon oil in papayas (Barrera-Necha et al., 2008). Two commercial plant oil-based fungicides have been recently labelled: Sporatec (Brandt Consolidated, Springfield, IL, containing rosemary, clove and thyme oils) and Sporan (EcoSmart Technologies, Franklin, TN, containing rosemary and wintergreen oils). However, a study on blueberry to control *A. alternata, B. cinerea* and *C. acutatum* diseases showed that only biofumigation with Sporatec resulted in significant reduction of *C. acutatum* disease; in addition, both biofumigants affected sensory qualities of treated blueberry negatively (Mehra et al., 2013). Some promising results in disease control of citrus fruit were found by incorporation of essential oils in wax coatings. Incorporation of *Lippia scaberrima* essential oil (2500 µL L^{-1}) in Carnauba Tropical coating (du

Plooy et al., 2009) or *Cinnamomun zeylanicum* (0.5%) in shellac and/or carnauba (Kouassi et al., 2012) led to a significant reduction of Penicillium disease in orange fruit, without detrimental effects on fruit. The advantage of using coatings amended with essential oils could be the close contact between the essential oils and the fruit surface over a long period.

1.3.3 Volatile organic compounds from microorganisms

Recently, increasing interest has also been devoted to biofumigation with VOCs produced by some microorganisms, such as *Muscodor albus* (Mercier and Jiménez, 2004; Mercier and Smilanick, 2005; Gabler et al., 2006), *Candida intermedia* (Huang et al., 2011), *Streptomyces spp.* (Wan et al., 2008; Li et al., 2010 and 2012) and *P. expansum* (Rouissi et al., 2013), showing fungicidal or fungistatic activity against a variety of postharvest pathogens. *M. albus* is anendophytic, non-spore producing fungus originally isolated from a cinnamon tree. Biofumigation with VOCs produced by *M. albus*, applied within 24 hours from inoculation, controlled brown rot on peach, grey mould on apple and table grape (Mercier and Jiménez, 2004; Gabler et al., 2006), blue mould on apple (Mercier and Jiménez, 2004) and green mould on lemon (Mercier and Smilanick, 2005), while only the treatment applied immediately after inoculation provided some control of sour rot on lemon (Mercier and Smilanick, 2005). Species of *Streptomyces* are gram-positive, filamentous and spore-forming bacteria. Volatiles of *S. globisporus* JK-1 (120 or 240 g L^{-1}) significantly reduced the incidence and severity of blue mould in Shatang mandarin (Li et al., 2010) and grey mould in tomato (Li et al., 2012). Although *P. expansum* is a common postharvest pathogen, VOCs emitted by *P. expansum* strainR82 completely inhibited the development of *B. cinerea*, *C. acutatum* and *M. laxa* (Rouissi et al., 2013). Many species of *Candida* yeasts are effective in fruit disease control and VOCs of *C. intermedia* C410, isolated from healthy strawberry leaf, were effective in reducing grey mould in strawberry (Huang et al., 2011). Some components of VOCs emitted from these microorganisms are common to different species of fungi, bacteria, yeasts or plants, whereas others seem to be unique for one species or isolate. The antifungal activity of these VOCs seemed to be not related to the relative abundance of the single components of the mixture. Geosmin, for example, is the primary component associated with the musty-earthy odour produced by many fungi and actinomycetes and it has been found to be the most abundant component also of *S. globisporus* JK-1; however, minor components (dimethyl trisulfide, dimethyl disulphide and acetophenone) of this mixture led to complete inhibition of blue mould in Shatang mandarin (Li et al., 2010). An advantage of the use of volatiles produced by microorganisms is that these VOCs occur as a mixture of compounds belonging to different chemical classes and these mixtures could have synergistic or additive properties that cannot be achieved by any single component alone. Phenylethyl alcohol is probably one of most effective

antifungal components, common to several microorganisms, including *M. albus*, *P. expansum* R82, *C. intermedia* and *S. globisporus* JK-1. However, when tested alone as a single component, it completely inhibited *B. cinerea*, *C. acutatum* and *M. laxa* myceliumgrowth and conidial germination at concentrations 2000 times higher than that naturally released from *P. expansum* R82 in culture (Rouissi et al., 2013). Moreover, the quantity and quality of VOC production by some microorganisms depend on the medium where they grow, as reported by Huang et al. (2012) for *Sporidiobolus pararoseus* strain YCXT3. The results suggested that *S. pararoseus* is incapable of accumulating highly toxic substances in NYDA and PDA media inhibitory to *B. cinerea*, while when it is cultured on yeast extract peptone agar it produced VOCs highly effective against both the conidial germination and the mycelial growth of the pathogen. Among 39 VOCs emitted by yeast and identified using GC-MS, authentic 2-ethyl-1-hexanol was found to have a strong antifungal activity against *B. cinerea*, suggesting that the strain YCXT3 of *S. pararoseus* is a promising agent for the control of grey mould disease under air-tight conditions. Disease control with VOCs produced by microorganisms is still at experimental levels and focused on antifungal activity, while the evaluation of effects on fruit quality or residues of treatment in fruit have not yet been carried out. These aspects should be taken into consideration, since the volatiles emitted from these microorganisms are characterised by strong odours (for example, the musty-earthy odour conferred by geosmin). The use of VOCs for fruit postharvest disease control on a commercial scale have also to be evaluated in relation to human safety, and more in-depth investigations on their toxicity for humans have to be performed. In fact, the occurrence of human asthma symptoms, for example, has been related to the emission of 2-ethyl-1-hexanol from dampness-related alkaline degradation of plasticiser floor material (Norback et al., 2000).

1.3.4 Chitosan

Chitosan is an edible coating derived from natural sources by deacetylation of chitin and considered harmless to humans and the environment; it has been studied for efficacy in inhibiting decay and extending the shelf-life of fruits (Aider, 2010). A large amount of data is available on the effectiveness of chitosan in pre and post-harvest treatments on fresh produce and recently reviewed by Shiekh et al. (2013). On temperate fruit like strawberries, a chitosan coating controlled Rhizopus rot and also reduced total aerobic count, coliforms, and weight loss of fruit during storage (Park et al., 2005). Similar results were obtained for small bunches of table grape dipped in 0.5% and 1% chitosan solutions. The treatment decreased the spread of gray mould infection from berry to the closed neighbours (nesting) (Romanazzi et al., 2002). The control of brown rot caused by *Monilinia* spp. was achieved in peach and cherry using the application of 0.1% and 1% respectively of chitosan (Li and Yu, 2000; Feliziani et al., 2013).

The infections caused by *R. stolonifer* on tomato fruits were inhibited after the application of chitosan, although the severity of soft rot symptoms was not related to the molecular weight of chitosan (Hernández-Lauzardo et al., 2011). *In vitro* trials showed the ability of chitosan to reduce mycelial growth of decay-causing fungi; the reduction is comparable with that obtained with synthetic fungicides (Feliziani et al., 2013). However, other results showed high contents of antioxidants, antioxidant activity, ascorbic acid, glutathione and high activity of β-1,3-glucanase in chitosan-treated strawberries proving a reinforced microbial defence mechanism of the fruit and an accentuated resistance against fungal invasion (Wang and Gao, 2013) .

1.3.5 Inducers of resistance

Several chemical compounds are known for their ability to induce disease resistance in treated plants, especially in weedy species, such as cotton (Colson-Hanks et al., 2012), and sunflower (Tosi et al., 1998), but also in woody plants, like grapevines (Reuveni et al., 2001). Recently, inducers of resistance have been tested directly on fruit in order to induce resistance against postharvest fruit pathogen.

β-aminobutyric acid (BABA), a non-protein amino acid, applied to specific wound sites on grapefruit peel surface is able to induce resistance against *P. digitatum* (Porat et al., 2003). Itsactivity is concentration-dependent, being most effective at the concentration of 20 mM, but less at higher or lower concentrations. BABA acts directly against pathogens; in fact, at increasing concentrations from 1 to 100 mM it showed a marked reduction in percentage of *P. digitatum* conidia germination and germ-tube elongation, in agreement with other data on *B. cinerea* and *P. expansum* on *in vitro* growth (Quaglia et al., 2011). However, when BABA was used as a postharvest treatment and fruit were dipped in the inducer solution, it had no effect or reduced the development of *P. italicum* and *P. digitatum* of orange only very slightly (Moscoso-Ramirez and Palau, 2013). Probably, when BABA is used with the aim of inducing resistance it does not reduce the percentage of infections or the lesion diameters; however, it can induce the activation of chitinase gene expression and protein accumulation (Porat et al., 2003). Similarly, acibenzolar-S-methyl (ASM) induced a significant increase in the levels of PR-1a (antifungal), PR-2 (b-1,3-glucanase) PR-5 (Thaumatin-like protein) and PR-8 (chitinase) gene transcripts in apple treated with increasing ASM concentrations (Quaglia et al., 2011). Salicylic acid (SA) is an endogenous hormone, having a key role in various species of plant growth. Positive SA effects have been reported for control of *P. expansum* in sweet cherry (Xu and Tian, 2008), gray mould decay in peach (Zhang et al., 2008) and fungal decays on persimmon fruit (Khademi et al., 2012). A stimulation of the antioxidant enzyme activities reported in sweet cherry fruit treated with SA (2 mM) suggested that the activation of antioxidant defence plays the main role in resistance against *P. expansum* (Xu and Tian, 2008).

However, the disease reduction obtained in treated fruit was very low (<15%) and SA cannot be considered satisfactory and recommended for inclusion in postharvest decay management programs for fruit packinghouses.

1.3.6 Integrated methods

All the alternative methods (physical and chemical) cited above did not always achieve an acceptable level of postharvest disease reduction when used individually, and some of these have a poor effect against future infection, after treatment or *vice versa* against established infections before treatment (Droby et al., 2009). To overcome this drawback, integrated strategies have been proposed and widely investigated. GRAS improved the efficacy of some microbial antagonists (Qin et al., 2006; Torres et al., 2007), although the effectiveness of combined treatments depends upon the mutual compatibility, duration and time at which they are applied. It is postulated that the enhancement of disease control is directly caused by the inhibitory effects of the salts on pathogen growth, and indirectly because of the relatively small influence of the GRAS on the growth of the antagonist (Qin et al., 2006). Physical methods like heat could enhance the bioefficacy of microbial antagonists such as *B. subtilis* (Obagwu and Korsten, 2003), *Pseudomonas syringae* (Conway et al., 2005), *Cryptococcus laurentii* (Zhang et al., 2007), providing in some cases an equivalent control to synthetic fungicides (Hong et al., 2014). The combination of HWD and ethanol improved the control of *M. fructicola* in peaches and nectarines compared to HWD and ethanol alone (Margosan et al., 1997). Similarly, a combination of peracetic acid and hot water showed a greater reduction of brown rot in 'Mountain Gold' and 'Rome Star' peaches inoculated with *M. fructicola* and treated with 40°C-heated peracetic acid (200 mg L^{-1}) than that observed in fruit treated only with hot water or peracetic acid (Sisquella et al., 2013). Recently, Dessalegn et al. (2013) demonstrated a high effectiveness in integrating plant defence inducing chemicals (PDIC), inorganic salt and hot water treatments for the management of postharvest mango anthracnose. Additive and synergistic increases in effectiveness were also observed by integrating heat therapy with various fungicides, thus leading to significant reductions in the application of active ingredients (Schirra et al., 2011). Imazalil (IMZ), a synthetic fungicide employed to control *P. italicum* and *P. digitatum* on citrus applied at 490 mg/L in aqueous solution at 37.8°C, was found more effective in decay control than IMZ in a wax mixture at 4200 mg/L sprayed on fruit at ambient temperature (Smilanick et al., 1997). On the other hand, the use of a fungicide in combination with hot water is not always preferable, since pyraclostrobin, a fungicide belonging to the anilinopyrimidine class, showed residues, in orange and lemons, greatly dependent on treatment temperature, approximately doubling for each 5°C increase in the mixture above 30°C (Smilanick et al., 2006). A possible synergistic effect between *Debaromyces hansenii*,an

antagonist yeast, and UV-C irradiation in controlling brown rot incidence on both artificially inoculated and naturally infected peaches was observed by Stevens et al. (1997). The superiority of the combined treatment (UV-C and yeast) was probably due to the ability of UV-C to control deep-seated infections such as a latent infections whereas the yeast controlled only superficial infections originating in recent wounds. The integrated treatment of gamma irradiation and a biocontrol agent (*Pseudomonas fluorescens*) on 'Golden Delicious' apple against blue mould allows instead the use of irradiation at low doses (200 and 400 Gy) with a dual benefit: no detrimental effects on fruit quality and significant control of disease (Ahari Mostafavi et al., 2013). Combined heat and UV treatment reduced postharvest decays, and maintained kumquat and orange quality. Heat treatment followed by UV-C radiation was the most effective combination, while if UV treatment preceded heat application, the elicitation of phytoalexins was inhibited (Ben-Yehoshua et al., 2005).

1.4 Conclusions

The microbial decay is one of the main factors that determines losses and compromises the quality of fresh produce, and although in the past the use of new fungicides has extended the shelf-life of fresh fruits, reducing losses, in the last decades, the concern about public health and the environment has considerably limited their use after harvest. Future scenarios are tending more and more towards integrated crop management and organic fruit production, with low use of fungicides, for a sustainable agriculture. This goal requires new technologies to control postharvest disease, arising from intense research that in the last 30 years has produced numerous studies. The results obtained show some significant progress in the reduction of pesticide use and disease control, although some critical points have still to be considered. It is unrealistic to assume that the physical and chemical methods described above have the same fungicidal activity as pesticides, and an integrated approach appears the best method to obtain acceptable results. However, further research is needed to investigate the activity of GRAS, natural compounds, VOCs, etc. in large-scale experiments, their mode of action and their degradation in organisms, which are still not fully understood. Physical methods probably have a better chance of prompt application on a commercial scale, since some of these, like heat, do not require any registration. Nevertheless, also in this case, more investigations have to provide additional information on appearance, texture, flavours and storability of treated fruit. Finally, research should lead to the development of appropriate tools (natural substances, GRAS, curing, heat, etc.) to tailor a complete integrated disease management strategy specific for each situation (species, climate and seasonal conditions, destination market, etc.).

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Control of Monilinia rots on fruit naturally infected by hot water treatment in commercial trials

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2.0 Abstract

In recent years, safer methods for the control of fruit postharvest pathogens have been investigated and heat treatment could represent an effective and safe approach for managing postharvest decay such as Monilinia rots. In the present study, the effect of hot water treatment (HWT) (60°C for 30 and 60 s) on brown rot was investigated. More specifically, the influence of HWT was determined in *in vitro* trials on conidial germination of *M. laxa*, *M. fructicola* and *M. fructigena* and in peach and nectarine fruit, naturally infected. The effect of hot water application on fruit quality was also assessed. *M. fructicola* showed a greater resistance to heat than *M. laxa* and *M. fructigena*, however conidia germination of all three species was completely inhibited by a dipping in hot water for 1 min at 55°C. The results of a large scale experiment under commercial conditions and several pilot trials showed a good antifungal activity of HWT in naturally infected fruit. In fact, after 6 days at 0°C and 3 days at 20°C, in both semi-commercial and commercial trials, the inhibition of decay was higher than 78% in four trials out of six. In addition, the treated fruit showed an acceptable commercial quality and no visual damage was observed as consequence of HWT. The results demonstrated that HWT is a promising method to control Monilinia rots of peach and nectarine, and is safe and readily available for conventional and organic production under commercial conditions.

Keywords: Heat treatment; Brown rot; *Monilinia* spp.; Peach; Commercial trials.

2.1 Introduction

Brown rot is an economically important disease in warm, humid climates areas where stone fruits are cultivated. The disease is mainly caused by *Monilinia laxa* (Aderhold and Ruhland) Honey and *Monilinia fructicola* (Winter) Honey, while *Monilinia fructigena* (Aderhold and Ruhland) is prevalent on pome fruits (Ogawa et al., 1995). In European countries, in the last century, only *M. laxa* and *M. fructigena* were isolated from fruit affected by brown rot while *M. fructicola* was considered a quarantine pathogen; however, following some studies that appeared in the last decade (Lichou et al., 2002; De Cal et al., 2009; Pellegrino et al., 2009), it was moved from A1 to A2 in the list of quarantine organisms (EPPO 2003). The pathogen is primarily a wound pathogen and in some cases, infections that occur in the field remain quiescent until the fruit reaches maturity, allowing *Monilinia* to overcome host defences. Eradication of these latent or incipient infections, which often develop within 24-48 h after harvest, is required. Consequently, marketing of decay-free fruit, especially to long-distance markets, can be problematic (Mari et al., 2009). The control of *Monilinia* rot depends on an integrated strategy based on cultural practices, orchard fungicide spray programme and, after harvest, on the maintenance of proper storage conditions in the packinghouse and during commercialization. Moreover, no chemical treatments are allowed on stone fruit after harvest in European countries (Jemric et al., 2011). Alternative strategies proposed for the control of Monilinia rots include treatments based on biocontrol agents (BCAs) such as yeasts (Mari et al., 2012), bacteria (Casals et al., 2010) and fungi (Mari et al., 2007), chemical products with low toxicity as food additives (Gregori et al., 2008), natural substances as biofumigants (Mari et al., 2008; Neri et al., 2007) or physical methods such as hot water treatments or modified atmosphere packaging (Karabulut and Baykal, 2004). A previous paper reported a significant reduction of *M. laxa* growth as conidia germination *in vitro* and as brown rot on peach and nectarine artificially infected after a water dipping at 48°C for 12 min (Jemric et al., 2011). Similar results were also obtained by Liu et al. (2012) on conidia germination and rot caused by *M. fructicola* using water at 40°C for 10 min. However, only few studies have evaluated this method under commercial conditions on naturally infected fruits. Laboratory and pilot-scale tests are essential to the development of treatment protocols, although optimal information can be found easily and quickly using small-scale experiments, it is important to transfer the pilot-scale or laboratory research results to large-scale industrial implementations.

The overall aim of the present study was to investigate the influence of hot water (HW) treatment on brown rot in naturally infected peaches. More specifically, the effect of HW treatment was determined in *in vitro* trials on conidial germination of *M. laxa, M. fructicola* and *M. fructigena* and *in vivo* on different cultivars of peach and nectarine cultivated under

conventional or organic management in laboratory and in semi-commercial and commercial-scale trials. Finally, the effect of hot water application on fruit quality was also assessed.

2.2 Materials and Methods

2.2.1 Pathogen

M. laxa,M. fructicola and *M. fructigena* strains were obtained from our collection, previously identified by sequencing of ribosomal DNA ITS regions (Mari et al., 2012) and maintained on potato dextrose agar (PDA) at 4 °C until use. In order to obtain a good sporulation of pathogens they were inoculated on V-8 agar (V8A: 250 ml of pure V8 juice and 40 g of agar in 1 L of distilled water) and incubated at 25°C with 12 h dark, 12 h light cycles for 10 days. Conidial suspensions were prepared by washing the colonies with sterile distilled water containing 0.05% (v/v) of Tween 80, quantified with a hemacytometer and diluted to the concentration of 10^6 conidia per ml.

2.2.2 Culturable conidia test

Conidia viability was measured as colony forming units (CFU) on PDA (Casals et al., 2010). Aliquots of 0.5 ml spore suspension (10^6 spores mL^{-1}) were added to 4.5 mL of water pre-warmed at 45°C, 50°C, 55°C and 60°C. Immediately after 1, 5 and 10 min of exposure, 0.5 ml of the warmed conidia suspension was diluted 100-fold in cold water. Aliquots (0.1 ml) of treated *M. laxa, M. fructicola and M. fructigena conidia* suspensions were spread on petri dishes and incubated for 3 days at 25°C. A suspension of untreated conidia (10^3 conidia per ml) was used as the control. Approximately 100 spores of each pathogen per treatment were evaluated by the culturable conidia test, expressing the results as number of CFU. The sample unit was represented by five plates (replicate) and the experiment was conducted twice.

2.2.3 Fruit

Peaches (*Prunus persica* (L.) Batsh) and nectarines [*P. persica* var. nectarina (Ait.) Maxim.] were obtained from a local packinghouse (Cesena, Italy) according to availability. Fruits free of visible wounds and rot and homogeneous in size were stored at 0°C and used within a couple of days after harvest. For laboratory trials, 'Caldesi 2010' nectarines and 'Benedicte', 'Royal Summer' and 'Symphony' peaches, cultivated in orchards under conventional management, were used; for semi-commercial and commercial trials, 'Royal Glory', 'Royal Mayestic', 'Red Moon' peaches derived from two orchards under organic management were used.

2.2.4 Influence of hot water treatment on brown rot in fruit with natural inoculum

Laboratory trials. Selected fruits were treated by dipping in a 10 L stainless steel tank. The water temperature was 60°C, the duration of treatment was 20 s. Control fruits were dipped in water at room temperature for the same time. After treatment, fruits were stored for 10 days at 0°C, followed by another 4 -7 days at 20°C (shelf-life). Disease incidence was recorded after refrigeration storage and after 4 days at 20°C, when the incidence of brown rot was low (< 5% in the control), fruits were kept for another 3 days at 20°C: the percentage of infected fruit was then evaluated for the second time. The sample unit was represented by 4 replicates of 25 fruit each per treatment and each trial was performed twice.

Semi-commercial trials. Plastic perforated boxes (51x31x26 cm) containing 60 fruits each were used for the semi-commercial trials. The HW treatment was carried out with the same parameters (temperature and duration) described above; a 50 L stainless tank was used for the treatment, the water was heated by a digital thermostat (ScanVac SHC 2000, Linge, DK) with temperature stability $\pm$ 0.01 °C, heater wattage 2 KW and pump having a flow rating of 15 L/min. Control fruits were dipped in the same tank containing water at room temperature. After treatments, fruits were stored at 0°C for 6 days and subsequently another 3 days at 20°C. The disease incidence was only recorded at the end of the experiment. The sample unit was represented by three replicates of one box each. The experiment was conducted twice.

Commercial trials. The commercial trials were carried out on peaches harvested from three different orchards and placed in bins, each containing 250 kg of fruit (1100-1200 fruits). A dipping machine (Xeda International, S. Andiol, France) was used for the treatment. The machine consisted of a tank containing 450 L of water, heated by electric resistances dipped in the water and regulated by a thermostat with temperature stability $\pm$ 1°C. The machine has a work capacity of 40 bins per hour. The treatment parameters of were set at 60°C for 60 s, since one minute is the minimum time allowing complete dipping of the bin, the subsequent treatment and the removal of the bin. Moreover, the top of the bin was covered with another empty bin, thereby ensuring that all fruits remained entirely submerged throughout the treatment. Prior to each HW treatment, the temperature of the bath was checked with a digital thermometer (HD8605, Delta Ohm, Padova, Italy) scaled to 0.1°C. Control fruits were treated in the same machine before water heating. After treatment, fruits were stored in the same conditions as the semi-commercial trials, as previously described. The disease incidence was only recorded at the end of the experiment. The sample unit was represented by three replicates of one bin each. The experiment was conducted twice.

2.2.5 Fruit quality

The effect of HWT on fruit quality was investigated on nectarine and peach fruit used for the laboratory trials. Visual fruit skin damage and physico-chemical parameters were evaluated at the end of each trial, after 10 days at 0°C followed by 4 or 7 days at 20°C, according to the experiment. Firmness (N, 8-mm fruit tester probe), soluble solid content (%) and titratable acidity (meq 100 mL^{-1}) were analyzed on 20 healthy fruits as reported by Neri et al. (2006).

The sensory profile was assessed by a trained panel after the period of shelf-life on fruit with the same firmness, taken from 20 fruits used for the instrument analysis as reported by Neri et al. (2007).

The sensory profile was determined using a 9-point category scale. The intensity of the sensory attributes increased from 1 (none) to 9 (extreme) for juiciness, mealiness, sweetness, sourness, fruitiness, off-odours and off-flavours. For hardness: 1: extremely hard and 9: extremely soft; for sweet-acid balance 1: mainly sour; 5: balanced and 9; mainly sweet; for colour: 1: green and 9: dark red.

2.2.6 Data analysis

The data from the experiments were combined based on Barlett's test which indicated homogeneity. All data regarding infected fruit were subjected to one-way analysis of variance (ANOVA) using the statistical package Statistica for Windows (Statsoft Inc.). Separation of means was performed using the least significance difference (LSD) test, at $P< 0.05$. All experiments were carried out in a completely randomized block design.

2.3 Results

2.3.1 Influence of hot water treatment on conidia germination

Conidia germination of all three species was completely inhibited after a dipping in hot water at 55°C for 1 min (Fig. 1). *M. fructicola* showed a greater resistance to heat than *M. laxa* and *M. fructigena* since no significant differences were observed in conidia viability (number of CFU) after 1 min of exposure at 45°, while *M. laxa* and *M. fructigena* conidia germination, in the same conditions, was significantly inhibited by 82% and 66.7% respectively. Complete inhibition of *M. fructicola* conidia germination was achieved by increasing the time of exposure (5 min at 50°C) or the temperature (55°C for 1 min).

2.3.2 Influence of hot water treatment on brown rot in fruits with natural inoculums

Laboratory trials. The antifungal activity of heat water was tested on naturally infected peaches and nectarines and stored at 0°C for 10 days followed by a shelf-life of 7 or 4 days. The low temperature inhibited brown rot, since very low percentages ($\leq$ 1.3 %) of infections were

detected on untreated peaches and nectarines after 10 days of refrigeration. When fruit were moved to shelf-life, the pathogen resumed and control fruit showed an incidence of brown rot ranging from 32.9 % ('Benedicte') to 85 % ('Symphony') (Table 1). At the first evaluation (after 10 days at 0°C), treated fruit showed a low percentage of infections, without any significant difference with respect to the control; after 4 ('Benedicte') or 7 days ('Caldesi 2010' and 'Symphony') of shelf-life, on the other hand, HW treatment significantly reduced the brown rot with an effectiveness index ranging from 34.4 % ('Symphony') to 87.2 % ('Benedicte').

Semi-commercial and commercial trials. In semi-commercial and commercial trials, the same cultivars were used. The duration of treatment was different for semi-commercial and commercial trials, 20 s and 60 s respectively. In the semi-commercial trials, at the end of storage, untreated fruits showed a *Monilinia* rot incidence ranging from 63.6 % in 'Royal Glory' to 44 % in 'Red Moon' with an average of 52.5% infection , higher than that observed in commercial trials (34.1 %), where 'Royal Glory showed the lowest percentage of brown rot (15.2%) and 'Red Moon' the highest (62.7%). However, HW treatments significantly reduced the disease in all trials, with an effectiveness index higher than 78% in four trials out of six and a similar average of disease reduction in semi-commercial and commercial trials (–75.8% and -78.2% respectively). The highest level of inhibition was obtained on 'Royal Glory' (-88.2%) and the lowest on 'Royal Majestic' (-58%), both in commercial trials. In all three cultivars, few fruit were affected by *M. fructigena* (5%) (data not reported) and the detection of the other two *Monilinia* species in all rotted fruit was considered too time consuming. Nevertheless, for each cultivar, some fruit showing Monilinia rot were analyzed by sequencing of ribosomal DNA ITS regions (Mari et al., 2012) and were found to be infected by *M. laxa* and/or *M. fructicola*.

2.3.3 Fruit quality

No visual symptoms of heat damage were observed in fruits treated with HW, even when fruits were exposed for 60 s to 60°C (commercial trial). Flesh firmness (about 50 N at harvest) decreased during cold storage (to about 29 N in 'Caldesi 2010', 35 N in 'Benedicte' and 42 N in ''Symphony' after 10 days at 0°C), but no significant differences were observed between treated and untreated fruit. Rate of softening further increased during shelf-life, reaching about 5, 6 and 7 N of firmness respectively, after 4 days at 20°C, but without significant differences between treated and untreated fruits. Minor modifications were observed for soluble solid content and acidity during storage and shelf-life, although no significant effects were induced by HWT on these fruit parameters (data not reported). No off-flavours or anomalous softening were observed by sensory evaluation in fruit treated with HW, nor were significant differences

found in any cultivar for sweetness, acidity, fruitiness, juiciness and hardness in treated fruit in comparison to the control (data not reported).

2.4. Discussion

Heat treatment of peach and nectarine fruits has been considered by many authors as a promising approach to control postharvest diseases such as brown rot (Karabulut and Baykal, 2004; Jemric et al., 2011) and storage apple rots (Maxin et al., 2012). However the information regard overall the laboratory experiments with artificial infected fruit and few data are available on the activity of heat against postharvest pathogens in fruit natural infected.

Our data showed, for the first in the same experiment, a completely inhibition of *M. laxa,M. fructicola* and *M. fructigena* conidia germination after exposure of 60 s at 55°C (Fig. 1). Similar results were previously obtained on *M. fructicola*, indeed 95% of spores were killed after 734 s at 46°C and after 206.3 s at 50°C (Margosan et al., 1997), while complete *M. fructigena* spore inactivation was achieved after a treatment at 43°C for 15 min or at 45°C for 5 min, although spores were inside a glass capillary dipped in a HW bath (Marquenie et al., 2002). In our study, *M. laxa* spore germination was reduced by 82% after exposure to HW (45°C) for 1 min and completely inhibited after 5 min at the same temperature (data not shown). In contrast, Jemric et al. (2011) obtained a reduction of *M. laxa* conidia germination of only 29.8% with respect to the control, after HWT at 44°C for 6 min. In order to evaluate the spore survival following fungicidal heat treatment, the number of germinated conidia, observed under the microscope, was normally used in *in vitro* trials (Margosan et al., 1997; Jemric et al., 2011). However, the germ tube elongation of conidia is not a good parameter to study the real vitality of conidia; the culturable conidia test, used in this work, and previously by Marquenie et al. (2002) and Casals et al. (2010), showing the capacity of conidia to produce or not a colony on agar medium, could better represent the inactivation of the pathogen after treatment. Our findings on *M. laxa* and *M. fructicola* conidia inhibition confirmed those obtained by Casals et al. (2010) and Karabulut et al. (2002) respectively, under *in vitro* conditions, using the culturable conidia test, and showing the complete inactivation of pathogen conidia exposed to 55°C for 1 min. The site of heat lethality was found in the nuclei and mitochondria of germinated spores of *Rhizopus stolonifer* (Ehrenb.:Fr. Vuill.) treated at 52°C for 2.5 min. (Baker and Smith, 1970); however, in the same work, spores of *M. fructicola* treated in the same conditions showed much less visible damage. The authors concluded that heat lethality could not be linked to the single event. More recently, Liu et al. (2012) observed intracellular reactive oxygen species (ROS) in *M. fructicola* spores after HWT at 40°C for 5 and 10 min. Since the levels of mitochondria damage are related to intracellular ROS accumulation

(Helmerhorst et al., 2001), they concluded that oxidative stress in *M. fructicola*, resulting in a collapse of mitochondria membrane potential and in a decrease of ATP levels, consequently delayed pathogen growth. Higher temperatures increased the efficacy of HWT, depending on the *Monilinia* species. *M. fructicola* appeared more resistant than the other two species and a greater capability to tolerate the ROS accumulation could be hypothesized for its heat resistance, although few data are available on oxidative stress in heat treated sporesof *M. laxa* and *M. fructigena*.

In preliminary *in vivo* trials, a fair inhibition of *M. laxa* with HWT below 60°C (data not reported) was obtained. These results confirmed previous works reporting water temperatures of 50°C and 55°C to be fairly effective against infections of *M. laxa* (Casals et al., 2010) and *M. fructicola* (Karabolut et al., 2010). The best condition of fruit treatment (water temperature and duration of dipping) was consequently set at 60°C for 20 s respectively. The acceptance of new disease control mean will depend on its effectiveness and compatibility with current handling and storage practices. More research is needed to determine the effects of various postharvest practices (such as washing, pre-refrigeration, storage, waxing, packaging, etc) on the efficacy of HWT, although a very short treatment (20-60 s) is optimal to accelerate fruit handling in packinghouses, normally adopted for fungicide treatments and better for commercial application than the longer treatments (6- 12 min) reported by Jemric et al. (2011). Peaches and nectarines are more resistant to heat than apple (Maxin et al., 2012) and treatment using a high temperature (60°C) for a short duration (20-60 s) was consequently preferred since it can be more easily integrated with handling practices. Previous results showed that HWT on fruit with natural infection was less effective than treatment on artificially inoculated fruit (data not reported)

In natural conditions, the inoculum could be represented by conidia on fruit surface or viable hyphae in latent infections that escape from the lesions by growing out between the cuticle and epidermis or just below the epidermal cells, as supposed by Wade and Cruickshank (1992) in apricots. While in the first case, conidia on the fruit surface were easily inactivated by HWT, the suppression of viable conidia or hyphae localized below the epidermidal cell appeared more difficult.

A low temperature (0°C) inhibited Monilinia rots in untreated and treated fruit; however, when they were moved to shelf-life, the pathogens resumed and high infection percentages occurred (Table 1). HWT was, however, effective and significantly reduced the rots, with a range of inhibition from -87.2% (‘Benedicte’) to -34.3% (‘Symphony’). The different activity of HWT could be attributed to the amount of conidia on the fruit surface and to a varying percentage of latent infections making it difficult to compare data on the efficacy of treatment.

On ‘Placido’ peaches, with natural inoculums, treated at 60°C for 40 s by dipping and stored for 21 days at 0°C plus 5 days at 20°C, the HWT reduced the incidence of brown rot by 25.5% (Casales et al., 2010). A high reduction of brown rot was obtained on naturally infected ‘Flavortop’ nectarines (-80.9%) by a hot water (60°C) brushing (Karabulut et al., 2002). This method involves rinsing fruit with a spray of hot water along the commercial packing sorting line and it has been adopted commercially for disinfection and cleaning of citrus (Porat et al., 2000), melon (Fallik et al., 2000) and other fruit and vegetables. The main advantage of a hot water immersion is a uniformly consistent temperature profile throughout the treatment tank at or slightly above the set point temperature, while in the case of hot water brushing (HWB) a possible non uniform coverage of fruit produced by hot water could be resolved in a limited pathogen control. HWB was found not quite as effective as HW dipping in apple trials (Maxin et al., 2012), although this technique has not yet been fully optimized. Although HWT requires specialized equipment, it might result in substantial economic saving since in the industrial packinghouse the heating of the water can be obtained from refrigerant gas (ammonia hydrochlorofluorocarbon, etc) coming from the cooling plant of the stores; indeed, the heat of this gas is usually dispersed in the environmental through the condensers.

At the end of trials, after 10 days at 0°C and 4 days at 20°C, the HWT did not affect fruit quality, indeed treated fruit maintained acceptable quality characteristics, without visual symptoms of heat damage. Fruit reached an acceptable stage for consumption (firmness between 5 and 12 N) (data not shown), as reported in other studies on quality of peaches (Jemric et al., 2011). Although a delay in fruit flesh softening was observed in fruit exposed to HWT and stored at low temperatures, probably due of inactivation of cell wall hydrolytic enzymes as polygalacturonase (Lurie, 1998) that quickly resumed when fruit were moved to shelf-life. On the same cultivar used in our work, the results relative to SSC and acidity parameters were similar to those previously obtained after a HWT at 46°C for 25 min by Malakou and Nanos (2005).

In semi-commercial trials, our findings showed a high activity of HWT on three different cultivars and similar results were found with the same cultivars, under the same conditions, also in commercial trials. The different duration of treatment in commercial and semi-commercial trials, 20 and 60 s respectively, does not seem to influence its efficacy. In both trials, the reduction of brown rot obtained by HWT was more than 50% with a maximum of 88.2% in ‘Royal Glory’ peach in commercial trials. To our knowledge, this is the first study demonstrating the activity of hot water in commercial trials against brown rot in naturally infected fruit. Taking into consideration that the used fruit came from organic production, our results acquire great importance since these productions can be prone to severe losses because

of the non-availability of chemical fungicides. However, also in conventional production, the concern about public health and the environment has considerably limited the use of traditional fungicides after harvest. In addition, HWT has a greater advantage than other non conventional control methods of fruit disease such as biological control agents and natural compounds, since it does not require any registration from the European Community, and the registration process for a biofungicide in Europe is more difficult than elsewhere, in terms of time and costs (Ehlers, 2011). In addition, HWT appears particularly attractive to the fruit industry since it could be immediately utilized and incorporated into handling practices before storage without extensive technical modifications.

The activity of HWT depends on at least two components, the first, as described above, is a direct and lethal action of heat on fungal inoculum as spores and mycelium present on the fruit surface or in the first layers of peel. The second component could be an indirect action of heat on the host, mediated by a stress-induced response of fruit (Maxin et al., 2012) including disease resistance. An indirect effect of HWT on *Penicillium digitatum* Pers. (Sacc.) was previously investigated in orange; HWT allowed the fruit to mobilize additional defense mechanisms against the pathogen growth by accumulation of phytoalexins (Nafussi et al., 2001), while proteomic analysis showed that HWT induced the over expression of defense-related genes such as chitinase and β-1,3-glucanase (Liu et al., 2012) or heat-shock proteins (HSP), in particular HSP70 (Rozenzvieg et al., 2004) in heat treated peach and grapefruit respectively. Furthermore, in apple subjected to HWT (45°C for 10 min), the HSP70 and HSP90 coding genes showed an up-regulation (Spadoni et al., 2012). However, the effective contribution of these compounds to pathogen control could be overestimated, and more investigations are required to elucidate the mode of action of the HWT on host-pathogen interaction. In conclusion, our results demonstrate the chance to effectively control Monilinia rots in naturally infected peaches and nectarines. The possibility to obtain a reduction of disease over 50%, in some cases up to more than 85%, without any detrimental effect for fruit quality, appears quite promising, considering that the results were obtained in commercial scale trials, very close to real operating conditions of a packinghouse.

2.5 Acknowledgments

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2.7 Figures

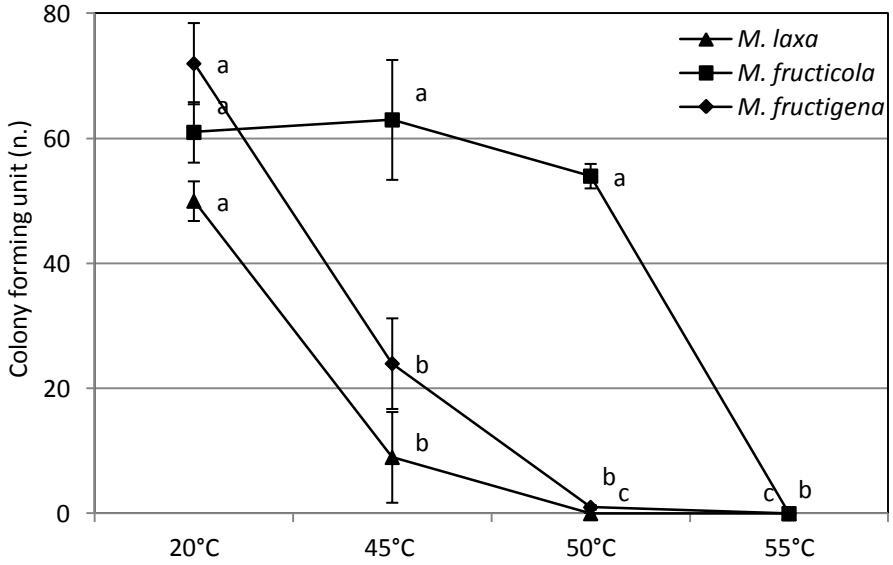

Figure 1 Culturable conidia of *Monilinia fructicola*, *Monilinia fructigena* and *Monilinia laxa* treated in a water bath at 20°C (control) and at 45°C, 50°C and 55°C for 1 min. The conidia were spread on Petri dishes and incubated at 25°C for 3 days. Each value is the mean of four replicates ± standard error. Within the same species the same letters represent no significant differences according LSD test (P<0.05).

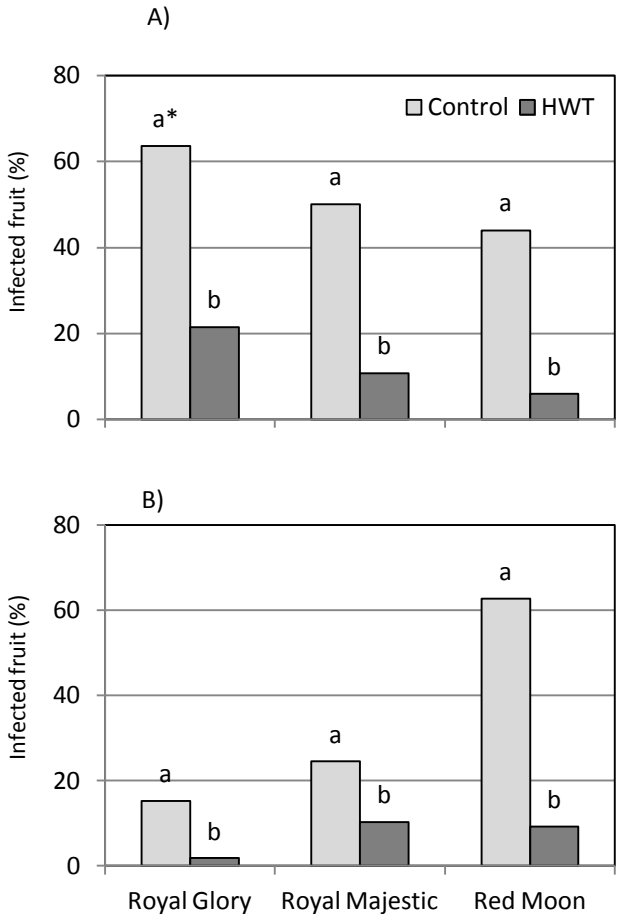

Figure 2 Effect of hot water treatment (60°C) against *Monilinia* rots on naturally infected peaches. A) Semi-commercial trials. The sample unit was represented by three boxes (replicate) of 60 fruits each. The duration of treatment was 20 s. B) Commercial trials. The sample unit was represented by three bins (replicate). The duration of treatment was 1 min. In both trials, after treatment fruits were stored at 0°C for 6 days and subsequently for another 3 days at 20°C. The brown rot incidence was recorded at the end of the experiment and the trials were conducted three times.

* Within the same trial and cultivar the same letters represent no significant differences according to LSD test ($P<0.05$).

2.8 Tables

Table 1

Effect of hot water treatment (60°C for 20 s) against *Monilinia* rots on naturally infected peaches and nectarines. Laboratory trial. After treatment fruits were stored at 0°C for 10 days, then at 20°C for 7 days ('Caldesi 2010' and 'Symphony') or for 4 days ('Benedicte'). Control fruits were dipped in water at room temperature for 20 sec. Each value is the means of 4 replicates of 25 fruits each.

Cultivar	After 10 days at 0°C		After shelf-life at 20°C	
	Control	HW	Control	HW
'Caldesi 2010'	0.9a*	0.8a	36.6a	20.7b
'Benedicte'	1.3a	0.8a	32.9a	4.2b
'Symphony'	0.4a	0a	85a	55.8b

* Within the same cultivar and in the same evaluation day, the same letters represent no significant differences according to LSD test ($P<0.05$).

Influence of hot water treatment on brown rot of peach and rapid fruit response to heat stress

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3.0 Abstract

Recent results on hot water as an alternative treatment seem to open a new perspective in disease incidence reduction. In the present work peach fruit were wounded, inoculated with conidia of *Monilinia laxa* and 15 min, 3, 6, 12, 24 and 48 h after inoculation treated by dipping in hot water (HT) at 60°C for 20 s. The effect of heat treatment on some cell wall genes involved in ripening such as *β-galactosidase* (*β-GAL*), *pectin lyase* (*PL*), *polygalacturonas e*(*PG*) and *pectin methyl esterase* (*PME*), was analyzed by qRT-PCR. The expression levels of defense related genes, *phenylalanina ammonia lyase* (*PAL*) and *chitinase* (*CHI*), heat stress-related genes as *heat shock proteins 70, 90* (*HSP70, HSP90*), and reactive oxygen species (ROS) scavenging genes was also evaluated by qRT-PCR. A 100% disease incidence reduction, as compared to untreated fruit, was obtained by treating 6 and 12 h after inoculation. Moreover brown rot was inhibited by 85.7% when fruit were heat-treated 48 h after inoculation. The expression levels of cell wall genes (*β-GAL, PL*,*PG* and *PME*),showed a general decrease in HT fruit as compared to the control, whereas *PAL, CHI, HSP70*and ROS-scavenging genes increased their expression level in HT samples with respect to the untreated ones. Our results show a curative activity of heat on peach inoculated with *M. laxa* 48 h before treatment. Each analyzed gene proved to be differentially expressed following heat treatment.

Keywords*:* heat treatment, postharvest diseases, stone fruit, gene expression, curative effect

3.1 Introduction

Brown rot is one of the main causes of losses in all stone fruit growing areas. The disease can be caused by three fungal species: *Monilinia laxa* (Aderhold and Ruhland) Honey, *Monilinia fructicola* (Winter) Honey and *Monilinia fructigena* (Aderhold and Ruhland). These pathogens infect blooms, twigs and fruit in the field, but severe damage more often occurs after harvest during storage and transport. Brown rot control depends on an integrated strategy based on orchard fungicide spray programs and cultural practices aiming to reduce the inoculum density and the infection risks. Nevertheless, these means are often inefficient and significant levels of Monilinia rots can develop during shelf-life, resulting in marked economic losses. Therefore, the interest for alternative control strategies is increasing because of the great concern for public health derived from the presence of chemical residues on fruit, the appearance of resistant isolates to common fungicides or their deregistration. Microbial antagonists, natural compounds or physico-chemical treatments have been evaluated (Droby et al. 2009; Nunes, 2012). However, one of the safest means is heat. Its efficacy against fungal pathogens, insect pests and some physiological disorders has, in fact, been known for a relatively long time and is well documented (Lurie, 1998; Fallik, 2004). Recent investigations reported a significant reduction of *M. laxa* incidence in fruit after a water dipping at 48°C for 12 min (Jemric et al., 2011); similarly, Spadoni et al. (2013) obtained a good reduction of brown rot in peach and nectarine infected naturally by dipping fruit at 60°C for 1 min. Heat can be applied to fruit in different ways: hot water dips or rinsing/brushing, vapour heat, anddry or wet (curing) hot air (Fallik, 2004). A very short (20-60 s) application is optimal to accelerate fruit handling in packinghouses, simulating fungicide treatments. Data from *in vitro* trials have suggested that heat is a fungicidal treatment, killing the conidia of *M. laxa* and *M. fructicola* after exposure (Spadoni et al., 2013). However, other modes of action cannot be excluded (Liu et al., 2012). It has, in fact, been reported that after a sub-lethal exposure to stress such as heat, fruit can develop resistance to other types of stress such as infection by pathogens (Ballester et al., 2010). It has been shown that abiotic and biotic stresses stimulate the activity of *phenylalanine ammonia-lyase* (*PAL*), the key enzyme of phenylpropanoid and flavonoid pathway, involved in the biosynthesis of important phenolics secondary metabolites, phytoalexins and lignins (Dixon et al., 2002). In particular, phenylpropanoid and flavonoid products play an important role as signal molecules, both in plant development and plant defense (Dixon et al., 2002). Abiotic stresses are well known to be involved also in the reactive oxygen species (ROS) production (Gill and Tuteja, 2010) which causes cellular damage resulting in oxidative stress. Consequently, the antioxidant defense system protects plants against oxidative stress, activating the main ROS-scavenging enzymes, such as ascorbate

peroxidise (APX), Mn-superoxide dismutase (MnSOD),glutathione reductase(GR), catalase (CAT) and NADPH oxidase (NADPH-OX). Among these, for example, *APX* transcription in banana fruit treated by hot water dipping (52°C for 3 min) was found to transiently increase at 1-6 h after treatment and decreased to the control level after 6 h (Wang et al., 2012). Previous works have also shown the ability of a heat treatment (HT) to promote the accumulation of heat shock proteins (HSPs), a conserved stress-responsive family of proteins accumulated in plants in response to high temperatures (Nover, 1991; Efeoğlu, 2009). *HSP90* showed inducible expression in response to heat shock at 44°C in apple fruit (Son et al. 2012), while Pavoncello et al. (2001) found that a hot water brushing (62°C for 20 s) treatment induced the accumulation of HSP70, HSP18 and HSP21 in grapefruit peel tissues. Softening is one of the main factors determining fruit quality and it can affect the onset of infections and physical injuries. It relates to texture changes as a consequence of modifications in polysaccharide component, through the disassembly of the middle lamella and primary cell wall structure (Brummell, 2006). These modifications involve the coordinated action of multiple cell wall degrading enzymes such as polygalacturonase(PG) and pectin methyl esterase (PME). The influence of HT on peach firmness was previously investigated without finding significant effects on fruit softening (Budde et al. 2006; Spadoni et al. 2013), although the inhibition of softening due to a reduced activity of cell wall degrading enzymes was reported in heat treated bananas (Amnuasyn et al., 2012). The overall aim of this research was to study the effect of HT on brown rot of peach fruit caused by *M. laxa*. More specifically, the following topics were investigated: i) the HT curative effect on fruit infected with *M. laxa,* and ii) the differential expression in HT and untreated peach fruit of ROS-scavenging genes (*APX*, *MNSOD, GR*, *CAT*, *NADPH-OX*), defense-related genes (*PAL*), heat-stress related genes (*HSP70* and *HSP90*) and cell wall genes (*PG, β-GAL, PL*, *PME*).

3.2 Materials and Methods

3.2.1 Pathogen inoculum

The *M. laxa* (ML4) isolate came from the collection of the Department of Agricultural Science, University of Bologna. It was previously identified by sequencing of ribosomal DNA ITS regions (Mari et al., 2012) and maintained on potato dextrose agar (PDA) at 4°C until use. In order to obtain a good sporulation, the pathogen was inoculated on V-8 agar (V8A: 250 ml of pure V8 juice and 40 g of agar in 1 L of distilled water) and incubated at 25°C with 12 h dark/12 h light cycle for 10 days. Conidial suspensions were prepared by washing the colonies with sterile distilled water containing 0.05% (v/v) Tween 80, quantified with a hemacytometer and diluted to the final concentration of 10^3 conidia/ml.

3.2.2 Fruit

Peach [*Prunus persica* (L.) Batsh cv 'Royal Summer'] fruit were cultivated in the experimental orchard of the Department of Agricultural Science, located in Bologna (Italy) (coordinates 44.559592°-11.410246°). The orchard was under conventional management, but no fungicide treatments against *Monilinia* spp. were performed. Fruit harvested at commercial maturity, free of visible wounds and rot and homogeneous in size, were stored at 0°C and used within a couple of days after harvest.

3.2.3 Curative effect of hot water treatment on M. laxa in artificially infected peaches

Selected fruit were wounded at the equator (one wound per fruit) with a sterile nail (2x2x2 mm) and inoculated with 20 µl of a *M. laxa* conidial suspension (10^3 conidia/ml). Fifteen minutes, 3, 6, 12, 24, and 48 h after inoculation, peaches were heat-treated (HT). Fruit were inserted in metallic grid baskets and submerged for 20 s in a stainless steel tank fitted with an electric resistance heater and a thermostat, containing water at 60°C. At the same time intervals, control fruit (C) were dipped in water at room temperature (20°C) for 20 s. After treatment all fruit were stored for 5 days at 20°C, and disease incidence (infected wounds, %) and severity (lesion diameter, mm) were recorded at the end of incubation. The sample unit was represented by 4 replicates of 6 fruits each, and the experiment was performed three times.

3.2.4 Data analysis

All data were subjected to one-way analysis of variance (ANOVA) using the statistical package Statistica for Windows (Statsoft Inc.). Separation of means was performed using the least significance difference (LSD) test at $P< 0.05$. All experiments were carried out in a completely randomized block design.

3.2.5 RNA isolation and qRT-PCR analysis

Total RNA from HT and control peach samples was isolated at different time points (0.25, 1, 3 and 6 h) after the heat treatment using Spectrum™ Plant Total RNA Kit (Sigma Aldrich, Madison, USA) following the manufacturer's instructions. Extracted RNA was treated with TURBO^{TM} DNase (Ambion, Austin, TX), visualized onanagarose gel to verify its integrity and quantified using ND-1000 UV spectrophotometer. For qRT-PCR experiments, first-strand cDNA was synthesized from 1 µg of total RNA in 20 µl reaction volume using ImPromII^{TM} Reverse Transcription System (Promega, Milan, Italy), following the manufacturer's instructions. The expression of each gene was normalized using *α-tubulin* as housekeepinggene. Primer pairs were designed using the Primer 3 program (http://bioinfo.ut.ee/primer3-0.4.0/) upon the sequences available in GenBank of the selected genes. The primer sequences, gene accession numbers and annealing temperatures are listed in Table 1.

Amplifications were run in a 96 well-plates MX3000 thermal cycler (Stratagene, La Jolla, CA, USA). In a total volume of 12.5 µl, each reaction mixture contained1X of Platinum Sybr-Green Master mix (Invitrogen, Milan, Italy), 0.5 µL of each primer (5 µM), 3.25 µL of nuclease-free water and 2.5 µL of 1:12.5 of cDNA. The following cycling conditions were used: an initial denaturation step at 10 min at 95°C, followed by 40 cycles consisting of 30 s at 94°C, an annealing step at a specific temperature (Table 1) and an extension step at 72°C for 1 min. Fluorescence was monitored at the end of each annealing step. To assess the amplification specificity, melting curve analysis was always performed at the end of each experiment, by monitoring the fluorescence from 55 °C to 95°C, every 0.1 °C. Quantification was carried out using the standard curve method (Applied Biosystem 2001). Standard curves were generated by serial dilutions of a randomly chosen cDNA first strand. Data were analyzed using MXPro QPCR Software version 3.0 (Stratagene, USA). For each sample, three independent biological replicates were made and each replicate was run twice. Means from independent experiments were subjected to one-way ANOVA.

3.3 Results

3.3.1 Curative effect of hot water treatment on M. laxa in artificially infected peaches

The effect of heat as curative treatment against *M. laxa* infections on wounded peaches is presented in Fig. 1. Brown rot was significantly controlled when peaches were immersed in hot water after inoculation, in comparison with control fruit. In particular, in control fruit, the lowest number of infected wounds was recorded 15 min after inoculation (50%), while the highest was at 3 h after inoculation (90%) (Fig. 1a). On the other hand, when fruit were heat-treated at 6 and 12 h after inoculation, the disease incidence reduction was complete, in comparison with control fruit (55 and 75% of infected fruit, respectively). When the time between heat treatment and pathogen inoculation increased to 24 and 48 h, brown rot was slightly reduced to 15 and 10%, respectively, as compared to control fruit (70%). A similar trend was observed as far as disease severity is concerned (Fig. 1b).

3.3.2 Effect of heat treatment on cell wall genes of peach fruit

Fig.2 reports the relative expression of *β-GAL*, *PG*, *PL* and *PME* genes analyzed by qRT-PCR. In our study, *β-GAL*, *PG*, and *PL* geneexpression at 15 min from treatment was found to be significantly (0.6-fold) lower in HT fruit as compared to control values. Three h after the treatment, the expression level of the same genes was also found to be 0.2, 0.4 and 0.5-fold lower in HT samples for *β-GAL*, *PG*, and *PL* genes, respectively, as compared to untreated fruit. A significant difference in *PME* expression between HT and control samples was evident only 3 h after treatment, and was 0.4-fold lower to that observed in control samples.

3.3.3 Effect of heat treatment on CHI, PAL,heat shock proteins genes in peach fruit

The gene expression of *CHI, PAL, HSP70,* and *HSP90* was also analyzed by qRT-PCR in HT and control fruit (Fig. 3 and 4). In HT samples, *CHI* expression was significantly down-regulated 3 and 6 h after the treatment while no significant differences in gene expression were found at 15 minutes and 1 h between control and heat treated fruit. On the other hand, at 15 min after treatment, the expression level of *PAL* was found to be 2.6-fold up-regulated with respect to the untreated samples (Fig. 3), whereas no significant differences were observed at the other time points.

The gene expression levels of *HSP70* and *HSP90* showed an opposite trend (Fig. 4). In particular the *HSP70* expression level was found to be significantly higher (3.0-fold) in HT samples, with respect to the control, at each sampling-time, but particularly at 1 h after treatment, when a 10-fold higher expression level in HT fruit than in control fruit was observed. On the other hand, a slight induction of *HSP90* at 1 h from treatment was observed in HT fruit as compared to the control.

3.3.4 Effect of heat treatment on ROS-scavenging genes in peach fruit

The expression of the ROS-scavenging genes as *APX, apx, cat, gr, mnsod,* and *nadph-ox,* was investigated in HT and control fruit using qRT-PCR. As in Fig. 5 our results show that the expression level of *APX* significantly increased in HT samples at all analyzed time points with respect to the untreated control. In particular, *APX* expression level was found to be 22 and 26-fold higher in HT samples compared to control values at 1 and 3 h, respectively, after the treatment. Also *cat, gr,mnsod,* and *nadph-ox* genes were found to be significantly over-expressed at 1 h after treatment: the expression levels were from 1.5 (*nadph-ox*) to 2.5-fold (*gr*) higher than those observed in untreated samples.

3.4 Discussion

In the present study, a curative activity of heat on peach inoculated with *M. laxa* was found. The treatment was effective against brown rot until 48 h after inoculation, reducing the incidence of disease by 85.7%. Several data are available on the control of postharvest fruit pathogens with a treatment after inoculation; treatments such as hot water, biocontrol agents, Generally Regarded as Safe (GRAS) compounds are often highly effective when fruit are inoculated just a few hours (1-2) before treatment, but the pathogens are not inhibited when the heat treatment is applied 48 h after pathogen inoculation (Sisquella et al., 2013). Indeed, Karabulut et al. (2010) kept inoculated peach fruit at 20°C for 12-16 h before heat treatment, in order to achieve spore germination within the wound sites and simulate the infections occurring during harvest; while Jemric et al. (2011) treated the fruit with hot water 24 h after

inoculation.However, to our knowledge this is the first time that hot water has been investigated as a curative treatment for peach brown rot performed at 48 h after inoculation. At 72 h after inoculation, the heat application couldn't be performed since the inoculated fruit showed lesions diameter already at 4-5 mm (data not shown). Obliviously, the temperature and duration of treatment can significantly affect the control effect (Spadoni et al., 2013). In natural conditions, the inoculum could be represented by conidia on the fruit surface or viable hyphae growing out between the cuticle and epidermis. Our data showed that the heat treatment (60°C for 20 s) was effective against both ungerminated or germinated conidia and hypahe at the first step of growth. The mode of action of hot water against brown rot is still under investigation but it is accepted that it is not linked to a single event (Margosan et al., 1997). Presumably, both a direct effect on the pathogen causing the disease and an indirect effect on the fruit host may be involved (Spadoni et al. 2013; Liu et al. 2012). Indeed it has been reported that heat treatment, performed at non-lethal conditions, causes moderate stress to fruit, provoking a temporary stop of the normal metabolism, that is recovered once the fruit is returned to a non stressing temperature (Paull and Chen 2000; Martinez and Civello 2008). The consequence is a delay in ripening and softening of treated fruit (Lurie, 1998). Heat treatments of strawberry fruit in an air oven for 3 h at 45°C showed a decrease in PG and β-GAL activity (Vincente et al., 2005); similarly, banana fruit after a water treatment at 50°C for 10 min, showed a reduced activity of PL and β-Gal enzyme whereas only a slight effect was observed on PME (Amnuaysin et al., 2012). Recently, Bustamante et al. (2012) demonstrated that peach fruit HT for 3 days at 39°C showed significantly lower expression levels of twelve cell wall modifying genes as compared to the control. The same treatment was applied on 'Dixiland' peach fruit and a transcriptomic profiling during the fruit postharvest phase showed a decrease in *PL* relative expression (Lauxmann et al., 2012). Our qRT-PCR results demonstrated a decrease in HT samples of cell wall gene expression at 15 min from treatment, as compared to untreated ones. In particular, the relative expression of *PME* and *PG*, encoding the most important enzymes acting on pectin polysaccharides, was significantly affected by the heat treatment. It has been reported that *PME* encodes a ubiquitous plant enzyme able to de-esterify to the methoxylated pectin present in the cell wall, releasing sites accessible to further degradation by PG (Vincente et al., 2005). Moreover, PG activity is responsible for solubilization and depolymerization of pectins during ripening. From our results, *PG* relative expression level was observed to decrease 0.6-fold in HT samples after 15 min and 0.5-fold 3 h after the treatment, while *PME* was down regulated in HT samples 3 h after treatment as compared to the control. Similarly, *PL* expression, which regulates the pectin degradation, significantly decreased soon after the treatment (15 min) and after 3 h by 0.6 and 0.5-fold as compared to the control, respectively.

Also *β-GAL*, involved in molecular signaling of pectin degradation, showed a decrease in expression level after heat treatment at all the analyzed time points. In mango fruit *β-GAL* is probably one of the key enzymes in pectin modification whose activity increases in parallel with increase in tissue softness during ripening (Ali et al., 1994). As reported by Amnuasyn et al. (2012) for banana fruit, a decrease in the relative expression of these cell wall genes in HT fruit might be correlated to a decrease in pectin solubilization and depolymerization, preventing pathogen infections. Indeed, it is widely reported that heat treatments are effective for delaying ripening in several fruit species (Lurie 1998; Paull and Chen 2000), contributing to reducing of fruit softening (Martinez and Civello, 2008) and better preparing peach fruit for a subsequent storage (Lauxmann et al., 2012) and avoiding or reducing the effect of some abiotic or biotic stress on fruit quality. In our study, a reduction in the expression of cell wall genes started soon after the treatment. The well-timed response might be the key to explain heat activity since it has been reported that in compatible plant–fungus interactions resistance mechanisms may be activated too slowly to be effective or may be suppressed by the invading pathogen (Sanzani et al., 2010).

The ability of heat treatment to induce stress responses has been widely investigated. Among genes involved in plant response upon biotic and abiotic stress, *PAL* has been suggested to play a crucial role in defense (Ferreira et al. 2007; Liu et al. 2012;). Indeed, *PAL* encodes for the first enzyme of the phenylpropanoid and flavonoid pathway and is thus involved in the biosynthesis of phenolics, phytoalexins, and lignins (Youssef et al., 2014). Moreover, *CHI* encodes for a pathogenesis-related (PR) protein and catalyses the hydrolysis of β-1-4-linkage of the N-acetylglucosamine polymer of chitin, which is an essential cell wall component of many fungal pathogens (Liu et al., 2012). In this study, HT fruit showed an immediate response to the treatment with the up-regulation (2.6-fold) of *PAL* gene 15 min after treatment as compared to the control. Pavoncello et al. (2001) observed that a hot water brushing treatment at 62°C for 20 s promoted the accumulation of chitinase content in the flavedo grapefruit 24 h after treatment, while in our study HT fruit showed a decrease in *chi* expression level with respect to the control at all considered times. This different behavior could be explained considering the two species, grapefruit and peach, and the times of analysis, 24 and 6 h after treatment, respectively. Moreover, Ballester et al. (2010) reported a low chitinase activity in the flavedo of cured orange fruit, thus suggesting that brushing as a stressing event might have contributed to the gene up-regulation.

At molecular level, the expression of heat shock genes encoding different HSPs is still poorly understood. It is known that they perform a critical function in refolding partially denaturated protein, completing degradation of denaturated proteins, assisting the *ex novo*

protein synthesis and averting protein aggregation (Aghdam et al., 2013). Our results showed a high level of *HSP70* expression in HT fruit from 15 min to 6 h after treatment, reaching a 10-fold induction at 1 h as compared to control samples, that might contribute to protect fruit against biotic and abiotic stress. Similarly, Promyou et al. (2008) demonstrated that hot water treatment induced high *HSP70* mRNA abundance in heat-treated banana fruit. Instead, an opposite trend was observed for *HSP90* that decreased in HT samples as compared to the control, in contrast with a previous study on *HSP70* and *90* gene expression in HT apple fruit (Spadoni et al., 2012), where *HSP90* gene expression level was up-regulated. Several correlations were found between the response to heat stress and the oxidative stress (Larkindale and Knight, 2002). For instance, Zhang et al. reported that hot air treatment (38°C for 10 h) on grape berries led to an increase in *HSP70* gene expression that was related to a synergic action with the berry antioxidant system (Zhang et al., 2005). In fact, it is known that heat shock may induce reactive oxygen intermediates causing membrane and protein damage (Paull and Chen, 2000). The accumulation of ROS in cells might activate the ROS scavenging pathways and result in the suppression of ROS in specific cellular locations or the entire cell (Mittler et al., 2004). In a previous work it was observed that antioxidant enzymes related to ROS metabolism during fruit ripening were regulated by heat treatment such as superoxide dismutase (SOD), peroxidases (POD) and catalases (CAT) (Paull and Chen, 2000). Vincente et al. (2005) reported changes in the oxidative metabolism of strawberry fruit following a heat treatment, with higher activities of Ascorbate peroxidase (APX) and SOD during storage. APX catalyses the conversion of H_2O_2 to water, with ascorbate serving as electron donor and thus plays a key role in regulating H_2O_2 levels and signaling in plant cells. In the present study, the expression of *APX* notably increased in HT fruit at all tested time-points, as compared to the control. A similar inductive effect of heat was reported on banana fruit by Wang et al. (2012) who demonstrated that the APX activities and correspondinggene expression in banana peel started to increase at 0.5 h after treatment and peaked at 1.5-6 h. H_2O_2 can be directly decomposed by combining APX with Glutathione reductase (GR), via recurrent oxidation-reduction reactions promoted by glutathione, hence preventing cell damage (Hung et al. 2005). In our work, in HT fruit *GR* gene expression increased after 1 h with respect to untreated samples. GR plays a key role as a detoxification enzyme by maintaining the intracellular glutathione pool in the reduced state to function as an important antioxidant, preventing ROS damage by scavenging free radicals (Yousuf et al., 2012). Moreover, *CAT* and *MNSOD* were found to be up-regulated in HT samples 1 h after treatment; this is in agreement with Maghoumi et al. (2013) showing that rinsed arils of pomegranate fruit, after hot water dipping for 30 s at 55°C, produced an initial increase in SOD and CAT activities as compared to the control. In addition to ROS scavenging

genes analyzed in our study, NADPH-OX showed a general expression decrease in HT fruit as compared to the control, except at 1 h after treatment, in accordance with the findings of Wang et al. (2012). This time-point corresponded with the highest relative expression of *apx*, *gr*, *cat* and *mnsod*, the major ROS-scavenging genes.

Our data showed that heat treatment completely controlled *M. laxa* infections 24 after inoculation and enhanced the expression level of *pal*, *hsp70*, *apx*, *mnsod*, *cat* and *gr* and, in the meantime, led to a slowing down of the cell wall genes mainly involved in ripening. These combined aspects might work together for a stress molecular response able to change the host-pathogen interaction during peach ripening. However, further studies are in progress to better understand the contribution of these genes to the defense response elicitation in fruit after heat treatment.

3.5 References

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3.6 Figures

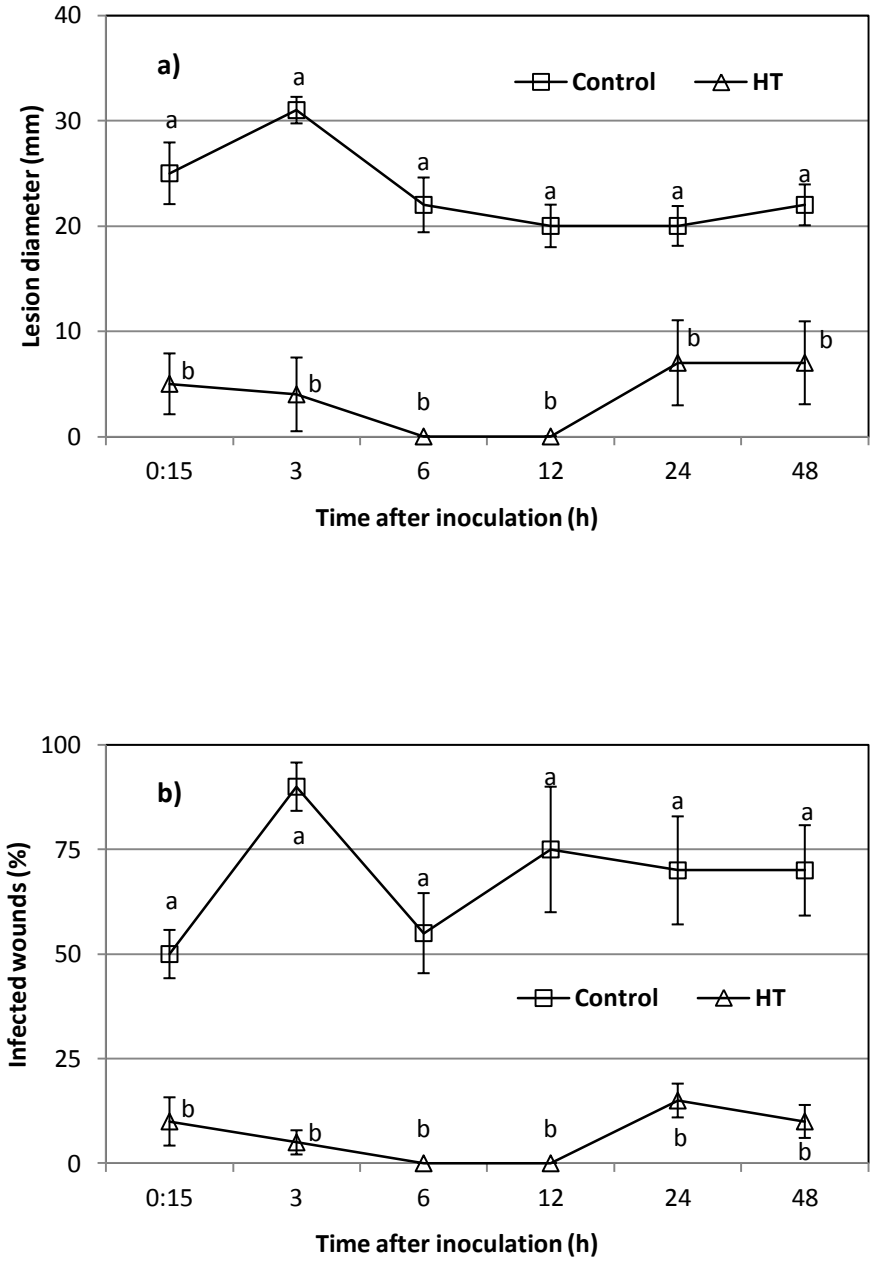

Figure 1 Effect of heat treatment on control of *Monilinia laxa* rot in peach fruit stored at 25°C. Severity (lesion diameter, mm) (a) and disease incidence (infected wounds, %) (b) were recorded 5 days after inoculation. Fruit were wounded, inoculated with pathogen (10^3 conidia/ ml) and after 15 min, 3, 6, 12 and 48 h treated by dipping for 20 sec in water at 60°C. Control fruit were dipped in water at 20°C for 20 sec. Each value is the mean of 4 replicates of 6 fruits each $\pm$ st er and within each time from inoculation, the same letters represent no significant differences according to LSD test ($P<0.05$).

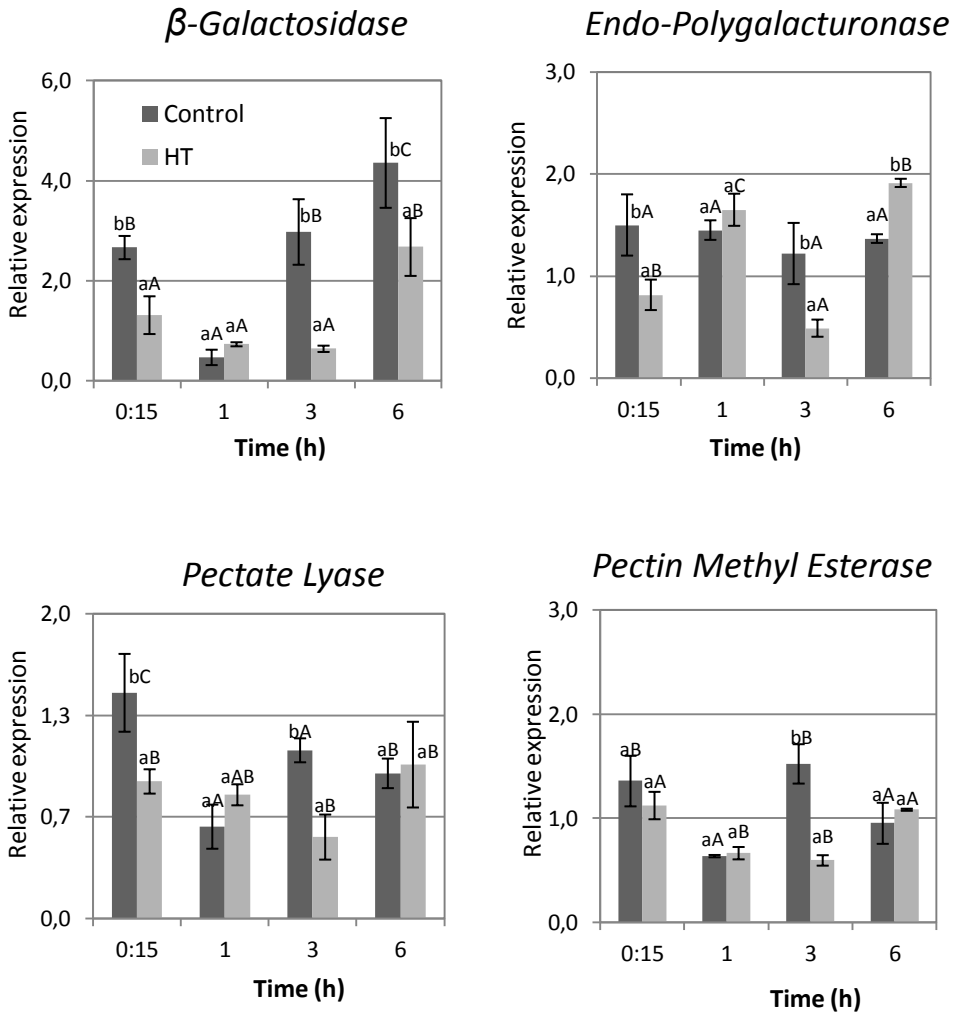

Figure 2 Relative expression levels of *β-gal*, *pg*, *pl* and *pmE* genes in heat treated and untreated peach tissues. Treated fruit were dipped in water at 60°C for 20 s whereas control fruit were dipped in water at room temperature for 20 s. Sample fruit were analyzed 15 min, 1, 3 and 6 h after treatment. All values were normalized using α-tubulin housekeeping gene. Each value is the mean of 3 replicates of 3 fruits each ± st dv. Within the same time point (lower-case) and within the time course (upper-case) the same letters represent no significant differences according to LSD test ($P<0.05$).

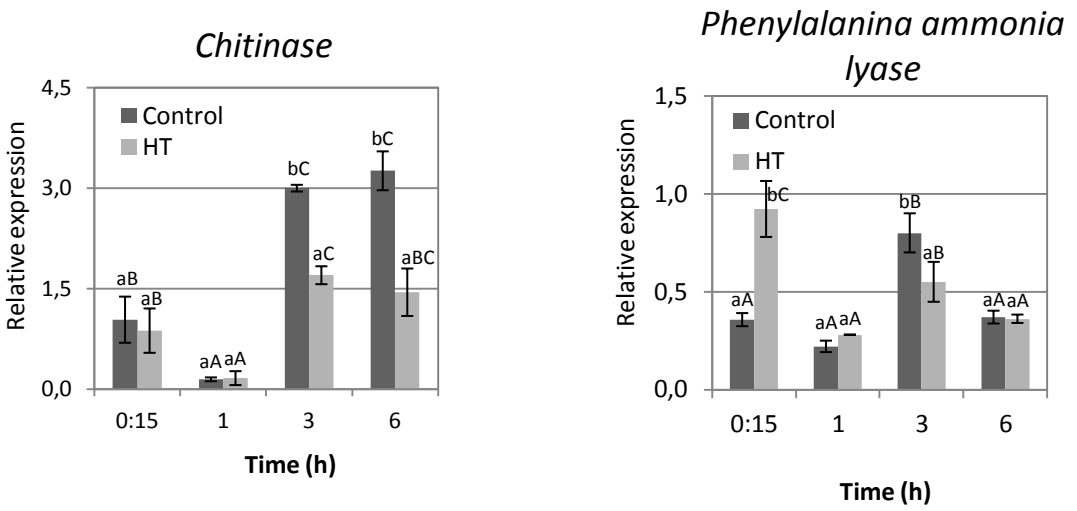

Figure 3 Relative expression levels of *CHI* and *PAL* genes in heat treated and untreated peach tissues. Treated fruits were dipped in water at 60°C for 20 s and control fruit were dipped in water at room temperature for 20 s. Sample fruit were analyzed 15 min, 1, 3 and 6 h after treatment. All values were normalized using α-tubulin housekeeping gene. Each value is the mean of 3 replicates of 3 fruit each $\pm$ st dv. Within the same time point (lower-case) and within the time course (upper-case) the same letters represent no significant differences according to LSD test ($P<0.05$).

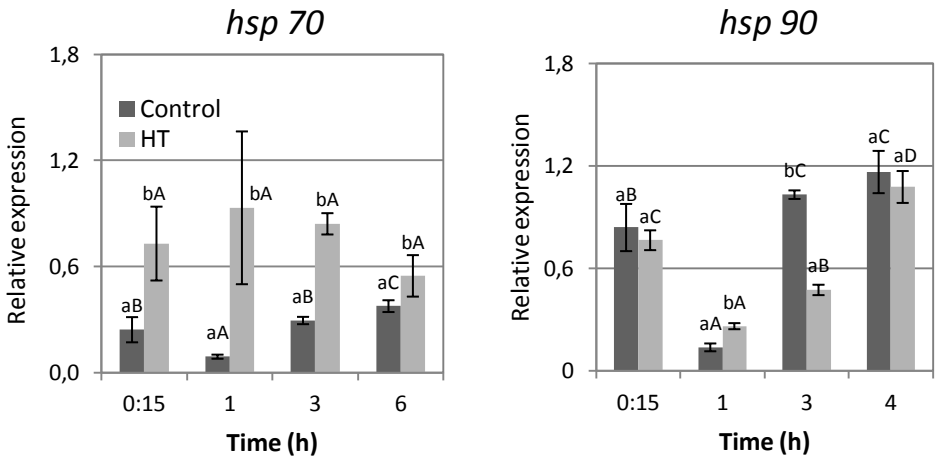

Figure 4 Relative expression levels of *HSP70* and *HSP90* genes in heat treated and untreated peach tissues. Treated fruit were dipped in water at 60°C for 20 s and control fruit were dipped in water at room temperature for 20 s. Sample fruit were analyzed 15 min, 1, 3 and 6 h after treatment. All values were normalized using α-tubulin housekeeping gene. Each value is the mean of 3 replicates of 3 fruits each ± st dv. Within the same time point (lower-case) and within the time course (upper-case) the same letters represent no significant differences according to LSD test ($P<0.05$).

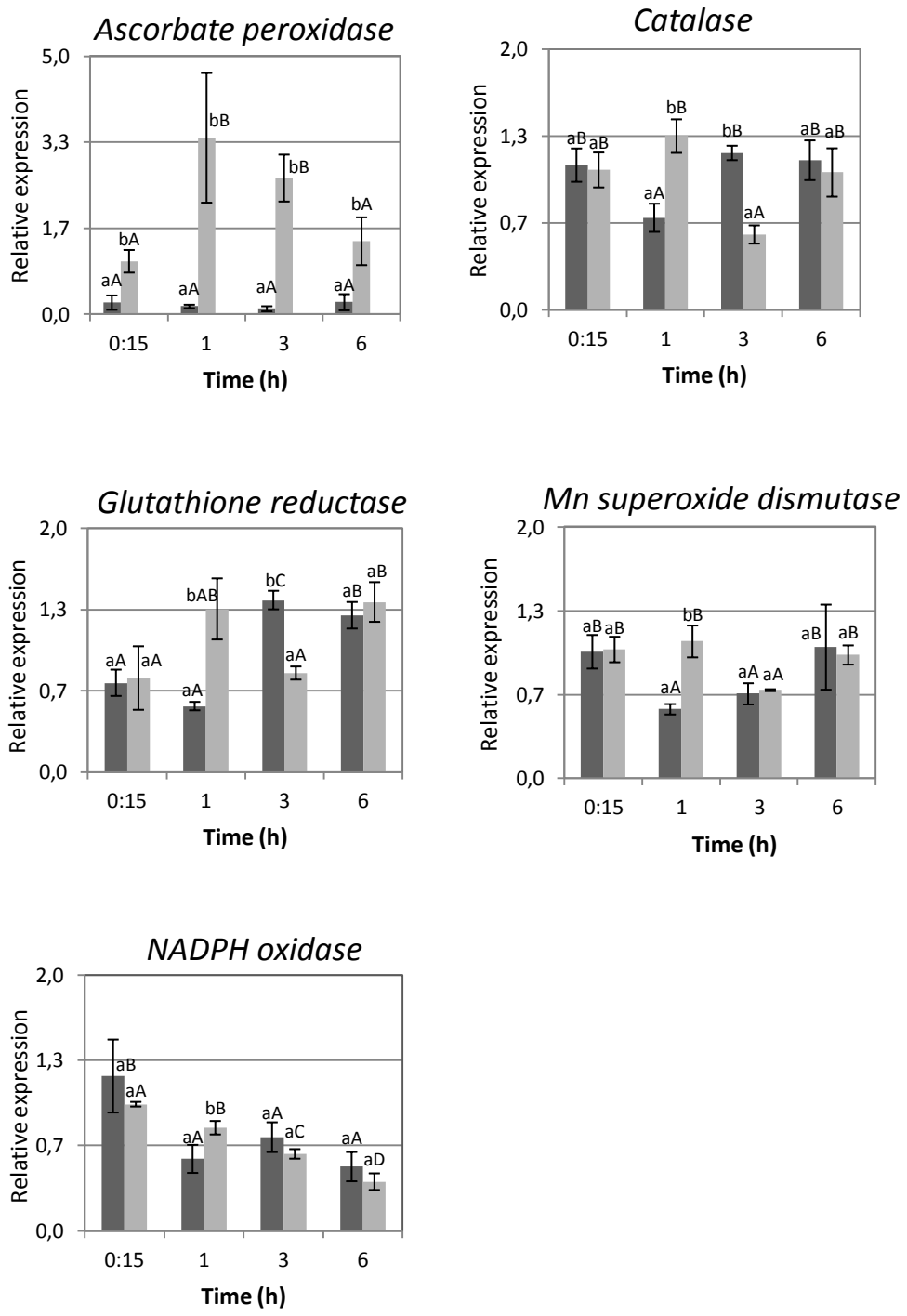

Figure 5 Relative expression levels of ROS-scavenging genes in heat treated and untreated peach tissues. Treated fruit were dipped in water at 60°C for 20 s and control fruit were dipped in water at room temperature for 20 s. Sample fruit were analyzed 15 min, 1, 3 and 6 h after treatment. All values were normalized using α-tubulin housekeeping gene. Each value is the mean of 3 replicates of 3 fruits each ± st dv. Within the same time point (lower-case) and within the time course (upper-case) the same letters represent no significant differences according to LSD test ($P<0.05$).

3.7 Tables

Table 1 Primers used in qRT-PCR reactions

Gene Name	Accession N°	Primer sequences (5'-3')	Annealing temperature (°C)
Ascorbato peroxidase	AF159630	F: CACAAGGAACGGTCTGGATT	57
		R: AAGGCCTTCCTTCTCTCCAC	
Catalase	AJ496418	F: GATCCTCGTGGTTTTGCAGT	57
		R: CGGGAATTTCATTCCATCAC	
Chitinase	AF206635	F: GAAAAGCAATAGGGGAGGA	57
		R: GACTGAGGGGTCATCCAGAA	
B-Galactosidase	EF568776	F: GTCTCAAGGCGGTCCAATAA	57
		R: TATGAGCTGCCCAGTCAGTG	
Glutathione reductase	JQ339738	F: GTGGTGGGGCTTAGTGAAGA	57
		R: CCGTCCGGATACAGTGTTCT	
Heat shock protein 70	DY637836	F: TGCGCAGAGGCAAGCAACA	57
		R: AATGTTCCACCACCAAGATC	
Heat shock protein 90	DY634477	F:TGAGCGTGAGCGTGGTATC	57
		R:CAAGGGTGAAAGCAAGAAGG	
Mn Superoxide dismutase	JQ364940	F: CGCCGTAGGTTCTACAGCTC	57
		R: CTGCATGATCTCGCCACTAA	
Phenylalanine ammonia lyase	AF206634	F: GTTGACCGCGAGTACGTTTT	57
		R: CCATTGTTCAATGCATGCTC	
Pectate lyase	AB264095	F: TGGAGGAATTGGAATTGGAG	57
		R: AAGCTTGAAGCCCTTGCATA	
Pectin methyl esterase	AB231903	F: TTCCTCCTCCAGTCTCTCCA	57
		R: TCCCTTTGCTTCTCAACGAT	
Endo-Poligalacturonase	DQ659241	F: ACAACATCGTGGTGAGTGGA	57
		R: GAAACCCTGACACCTTGCAT	
NADPH oxidase	JN791439	F: TTGGCTACATTGAGCTGTGG	57
		R: GCTTAGAGCTTGGCTCGTGT	
α-Tubulin	DY650410	F: GGTGGTGGTACTGGTTCTGG	57
		R:CTGCGGAGAAGGATAGATGG	

Controlling *Monilinia* rot by heat treatment? The role of peach VOCs

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4.0 Abstract

Heat treatment (HT) represents one of the safest methods for the control of fruit postharvest disease such as *Monilinia* rot. The present study focuses on the development of the method considering the involvement of VOCs. HT applied as preventive method by dipping the fruits in warm water at 60°C for 60 s stimulates rot severity. Control artificially inoculated fruits showed a severity reduction ranging from 21,6% to 8,4% when inoculation was performed respectively after 1 and 3 h from treatment. Moreover, microscope investigations showed that *M. fructicola* germ tube length was three times higher (135 µm) on peach fruit skin heat treated and inoculated after the treatment compared to the control (58 µm). In a preliminary assay VOCs produced by HT fruit stimulated the conidia germination of 64% in 'Springbelle' peach, of 40% in 'Red Haven' peach and of 33% in 'Lucie Tardibelle' peach. In order to identify the VOCs produced by heat treated peaches solid-phase microextraction/gas chromatography - mass spectrometry (SPME/GC-MS) and proton transfer reaction - time of flight - mass spectrometry (PTR-ToF-MS) were used. An increase in the emission of acetaldehyde and ethanol by fifteen and twenty eight folds, respectively, was detected in HT peaches cv. 'Lucie Tardibelle' compared to unheated ones. In order to validate these data, pure compounds were tested on *M. fructicola* conidia germination and mycelia growth. Our results showed a stimulatory effect of acetaldehyde (0.6 µL L^{-1}) and ethanol (0.2 µL L^{-1}) treatment on *M. fructicola* conidia germination by 40% and mycelia growth by 6 mm, respectively.

4.1 Introduction

Peaches are climacteric fruits which deteriorate quickly at ambient temperature due to rapid ripening process and to abiotic and biotic stresses. Within microbiological alterations, brown rot caused by *Monilinia* spp. is one of the main diseases affecting stone fruit and limiting the extension of fruit storage life (Sisquella et al., 2013). *Monilinia* spp. penetration is favored by the occurrence of wounds and physical damages. In some cases the infections, occurred in the field, remain quiescent until fruit reaches ripeness, allowing pathogen to overcome host defences (Mari et al., 2009). Generally, when field conditions are favorable for disease

development, the losses during storage can be important, reaching high values (59%) (Larena et al., 2005). The control of *Monilinia* rot depends on an integrated strategy based on cultural practices and fungicide spray program in the field, since postharvest treatment with fungicides are not allowed in European countries. In the last decades, the interest in "organic" and "safe" food products increased in the consumers, inducing the evaluation of more environmentally friendly postharvest treatments to maintain high fruit quality and reduce losses during storage. Among the various non-conventional approaches tested, heat treatment (HT) is completely safe and have shown high efficacy in the control of postharvest diseases, either when applied alone (Bustamante et al., 2012; Li et al., 2013; Spadoni et al., 2013) or combined with peracetic acid (Sisquella et al., 2013), low toxicity salts (Cerioni et al., 2013) and antagonistic yeasts (Zhao et al., 2009) showed a good efficacy in reducing rots. Most studies in literature have proved the curative activity of HT against fungal diseases. Karabulut et al. (2010) showed that brief hot water treatments applied to fruit inoculated with *M. fructicola* and incubated at 20°C significantly controlled brown rot. In our previous study, dipping in hot water at 60°C for 60 s of peach naturally infected significantly reduced the development of brown rot in commercial conditions (Spadoni et al., 2013), in addition, a good curative activity was observed on peach inoculated and treated by heat (60°C for 60 s) until 48 after inoculation with *M. fructicola* (Spadoni et al., in press). Similarly, heat was curative for the anthracnose of strawberry (Vincente et al, 2005) and the bull's eye rot of apple (Neri et al., 2009). The primary mode of action of HT is killing or damaging of infection structures of fungi present on the fruit surface or in the first layers under the skin (Palma et al., 2013; Yun et al., 2013), and a collapse of mitochondrial membrane potential of *M. fructicola* spores after heat treatment was observed in peaches (Liu et al., 2012). The preventive activity of HT in disease control has been less studied, however, the increasing of the enzymatic activity of enzymes responsible of the host defense mechanism activation after HT has been also reported (Liu et al., 2012). The occurrence of detrimental effects on fruit physiology could be possible disvantages of use of HT, and temperature lesser than 65°C are generally applied on peaches to avoid physical injury. However few studies have focused on effects on HT on peach metabolism. In particular, the influence of heat on fruit volatile emission has been not widely studied, as well as the influence of fruit volatile organic compounds (VOCs) on fungal pathogen growth.

The aims of the present work were to investigate on i) the preventive effects of a HT on *M. fructicola* of peach; ii) the direct effects of VOCs emitted by heat treated peaches on *M. fructicola* growth by *in vitro* and *in vivo* trials, and iii) the VOCs production by heat treated detect with solid-phase micro-extraction/gas chromatography - mass spectrometry (SPME/GC-MS) and with proton transfer reaction - time of flight - mass spectrometry (PTR-ToF-MS).

4.2 Materials and methods

4.2.1 Fruit

Yellow-fleshed 'Springbelle', 'Royal Summer', 'Red Haven' and 'Lucie Tardibelle' peaches (*Prunus persica* L.) were grown in commercial orchards located in Emilia Romagna (Italy) following standard cultural practices and chemical spray programs, while no fungicide treatments against *Monilinia* spp. were performed. Fruits without visible wounds or rots and homogeneous in size were harvested at commercial maturity, stored at 0°C and used within a couple of days after harvest.

4.2.2 Pathogen

The isolate of *M. fructicola* (MFA), obtained from our collection and previously identified by sequencing of ribosomal DNA ITS regions (Mari *et al*., 2012), was maintained on potato dextrose agar (PDA) at 4 °C until use. In order to obtain a good sporulation of pathogen, it was inoculated on V-8 agar (V8A: 250 ml of pure V8 juice and 40 g of agar in 1 L of distilled water) and incubated at 25°C with 12 h dark, 12 h light cycles for 10 days. Conidial suspensions were prepared by washing the colonies with sterile distilled water containing 0.05% (v/v) of Tween 80, quantified with a haemocytometer and diluted to the useful concentration for each assay.

4.2.3 Influence of a preventive hot water treatment of fruit artificially infected with **M. fructicola**

The effect of preventive treatment with hot water to control *M. fructicola* infections was assayed on unwounded and wounded 'Red Haven' peaches. Fruit, were previously washed with sodium hypochlorite (1% v/w) and rinsed in distilled water for 1 min to remove pathogen natural inoculum from the surface and left to dry at room temperature for 1 h. Successfully, a lot of unwounded fruits were heated using the methodology proposed by Spadoni et al. (2013) with slight modifications. Peaches were inserted in metallic grid baskets and submerged for 60 s in a stainless steel tank fitted with an electric resistance heater and thermostat containing water at 60°C. Control fruits were represented by fruits dipped in water at room temperature (20°C) for 60 s. After 0, 15 minutes and 1, 3 and 6 h from treatment all fruits were inoculated by dipping in a *M. fructicola* spore suspension ($5x10^3$ conidia mL^{-1}) and kept for 5 days at 20°C. Subsequently, the percentage of infected fruits was recorded. The sample unit was represented by 4 replicates of 6 fruits each and the experiment was performed twice. Another lot of fruits, previously disinfected, were wounded with a sterile nail (2x2x2 mm) and heated as previously described. Then after 0,15 minutes and 1, 3 and 6 h from treatment fruits were

inoculated introducing 20 µl of a *M. fructicola* conidia suspension (10^3 conidia mL^{-1}) into the wound. Peaches wounded, dipped in water at 20°C and inoculated as cited above represented control fruits. All fruits were kept for 5 days at 20°C and the lesion diameter (mm) was recorded. The sample unit was represented by 4 replicates of 6 fruits each and the experiment was performed twice.

4.2.4 Effect of heat treatment on **M. fructicola** *germ tube elongation on fruit surface*

The *M. fructicola* conidia germination on the surface of heat treated peaches was investigated by microscopic observations. For this purpose 'Red Haven' peaches were sterilized and treated by heat as described above and after 0 (T0) and 24 h (T24) from treatment were sprayed with a 10^6 conidia mL^{-1} of *M. fructicola* for 1 min by a hand-sprayer pipette distributing an aliquot of 5 mL per fruit. Control fruits were represented by fruits immersed in water at room temperature and inoculated as described. After inoculation, the fruits were placed in separate glass boxes (32x24x20 cm) covered on the bottom with a paper leaf, soaked with 10 ml of distillated water keeping an high relative humidity (>95%) and the boxes were incubated at 20°C. To observe the germ tube elongation of *M. fructicola* conidia present on fruit surface, at 18 h post-inoculation (hpi), a fruit epidermal layer (4x4 mm) was removed with a scalpel to a depth of about 0,5 mm in three different positions per fruit. The tissue pieces were placed on a glass microscope slide; the conidia were stained with 1–2 drops of lactophenol blue solution (Sigma Aldrich, Milan, Italy) and visualized on a Nikon Eclipse TE2000-E as described by Guidarelli et al. (2011). The germ tube of *M. fructicola* was measured at 18 hpi in both conditions (T0 and T24). The sample unit was represented by 3 microscope observations (replicates) of 10 germ tubes each per both inoculation times (T0 and T24).

4.2.5 Influence of VOCs emitted from heat treated peaches on **M. fructicola** *conidia germination*

A pathogen conidia suspension (10^3 conidia mL^{-1}) was prepared as described above and an aliquot of 100 µL was spread on malt extract agar (MEA) Petri dishes. "Springbelle", "Red Haven" and "Lucie Tardibelle" peaches were heat treated by dipping in water at 60°C for 60 s. Immediately after the treatment, fruits were placed inside the glass boxes described above. The plates inoculated with *M. fructicola*, opened and overturned to expose directly the plate surface to VOCs produced by fruit, were inserted in the same boxes. A mesh was used to avoid any physical contact between pathogen and fruit surface. Control was represented by boxes containing fruit immersed in water at room temperature. In each box there were 10-12 peaches per cultivar, depending on fruit size and 8 inoculated plates, boxes were sealed and incubated at 20°C for 4 days. The conidia viability was measured as colony forming units (CFUs).

4.2.6 Analysis of volatile organic compounds emitted by heat treated fruit

Proton transfer reaction - time of flight - mass spectrometry (PTR-ToF-MS) analysis. VOCs emission by 'Lucie Tardibelle' peach fruit was analyzed following the procedure described in previous works for other food samples (Soukoulis et al., 2013) using a commercial PTR-ToF-MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria) in its standard configuration (V mode). For the analysis, each single fruit was placed in a glass jar (1000 mL) provided with two Teflon/silicone septa on opposite sides. Before measurement HT peach fruits were immersed in a water bath for 1 minute at 60°C while control peach fruits were immersed in water at room temperature for 1 minute. To standardize the measurements, all samples were then equilibrated at room temperature for 30 min prior to analysis. Peach fruits were consequently closed hermetically for 10 min inside the glass jars to accumulate the volatile compounds emitted. VOCs were then measured by direct injection of the head space mixture into the PTR-ToF-MS drift tube via a heated (110°C) peek inlet for 10 min. The sampling time per channel of ToF acquisition is 0.1 ns, amounting to 350000 channels for a mass spectrum ranging up to m/z = 400, with the following conditions in the drift tube: drift voltage 600 V, temperature 110°C and pressure 2,25 mbar. Every single spectrum is the sum of 28600 acquisitions lasting for 35 μs each.

Spectra analysis. The external calibration automatically done by the acquisition program provided a poor mass accuracy, thus internal calibration of ToF spectra was performed off-line (Cappellin et al., 2010). Signal losses caused by the detector dead time and duty cycle were corrected for (Cappellin et al., 2011). Data pre-processing on ToF spectra was carried out to remove the baseline and noise reduction was achieved by averaging over the 30 consequent ToF spectra corresponding to the same sample, thereby allowing the improvement of the signal-to-noise ratio by about five times. For peak identification and area extraction the procedure followed was of Cappelin et al. (2011). The experimental *m/z* values reported were up to the third decimal. VOC concentrations are expressed in ppbv (part per billion by volume) and have been calculated from peak areas according to Lindinger et al. (1998), using tabulated values for the reaction rate coefficient (Cappellin et al., 2012). When the reaction rate coefficient was not available within the literature, a constant value ($k = 2\ 10^{-9}$ cm^3/s) was employed. *SPME/GC-MS analysis*. For the SPME/GC-MS analysis a similar procedure than the one employed for the PTR-ToF-MS analysis was used. Before measurement, control peach fruits were immersed in water at room temperature for 1 minute while heat treated (HT) peach fruits were immersed in a water bath for 1 minute at 60°C. To standardize the measurements all samples were equilibrated at room temperature for 30 min prior to analysis. Each single fruit was placed in a glass jar (1000 ml) provided with two Teflon/silicone septa on opposite sides and kept at room

temperature for 30 min for volatile compound collection. Headspace volatile compounds were collected by a 2 cm Solid Phase Microextration fibre coated with divinylbenzene/carboxen/polydimethylsiloxane 50/30 lm (DBV/CAR/PDMS, Supelco, Bellefonte, PA, USA), inserted thorugh a Teflon/silicone septum using a manual holder (Supelco, Bellefonte, PA, USA). The fibre was exposed to the peach headspace for 30 min. Volatile compounds adsorbed on the SPME fibre were desorbed at 250°C in the injector port of a GC interfaced with a mass detector operating in electron ionization mode (EI, internal ionization source; 70 eV) with a scan range from *m/z* 35–300 (GC Clarus 500, PerkinElmer, Norwalk CT, USA). Separation was achieved on a HP-Innowax fusedsilica capillary column (30 m, 0.32 mm ID, 0.5 lm film thickness; Agilent Technologies, Palo Alto, CA, USA). The GC oven temperature program consisted in 40°C for 3 min, then 40 – 220°C at 4°C min^{-1}, stable at 220°C for 1 min, and then 220 –250 at 10°C min^{-1}, and finally 250°C for 1 min. Helium was used as carrier gas with a constant column flow rate of 2 mL min^{-1}. Compound identification was based on mass spectra matching with the standard NIST05/Wiley98 libraries and retention indices (RI) of authentic reference standards.

4.2.7 Effect of pure volatile organic compounds on **Monilinia fructicola** *growth*

The effect of ethanol and acetaldehyde, the main VOCs emitted by heat treated peaches, was assayed against the conidial germination and mycelium growth of *M. Fructicola.* Aliquot of 100 ml of a conidia suspension of *M. fructicola* or a plug (6-mm diameter) from an actively growing pathogen culture were respectively spread or placed in the centre of MEA plates. In each case, aliquots of 0.2 µL L^{-1} of pure ethanol or 0.6 µL L^{-1} of pure acetaldehyde, were placed, using a microsyringe, on a paper filter (Whatman no. 1, 90-mm diameter) positioned inside the cover, as described by Neri et al. (2009). VOC concentrations were selected in order to roughly match the corresponding concentrations derived from VOC emission by HT fruits determined with PTR-ToF-MS (see the result section). The dishes were quickly closed, sealed with Parafilm, and incubated at 25°C. Control samples were represented by Petri dishes inoculated with pathogen but treated with distilled water instead of a chemical compound. The CFUs were recorded after 48 hours of incubation, while the mycelium growth was recorded after 5 days as colony diameter (mm). For each compound tested and for each biological fungal parameter three Petri dishes (replicates) were used. The assay was performed three times.

4.2.7 Statistical analysis

All data regarding infected fruits, culturable conidia test and germ tube elongation were subjected to one-way analysis of variance (ANOVA) using Statistica for Windows (Statsoft

Inc.). Separation of means was performed using the least significance difference (LSD) test, $P < 0.05$. For the statistical analysis of VOC data specific scripts were developed using R (R foundation for statistical computing, Vienna, Austria). Pair wise multiple comparisons were made using the post-hoc Tukey test at the same significance level. All experiments were carried out in a completely randomized design.

4.3 Results

4.3.1 Influence of a preventive hot water treatment of fruit artificially infected with M. fructicola

Hot water treatment applied before inoculation with *M. fructicola* failed to control the development of brown rot in peaches artificially inoculated with the pathogen, both in unwounded and wounded fruits. In unwounded fruits, the incidence of infection in heated fruits was 100%, for all times of inoculation tested, while in unheated fruits the incidence of disease was significantly lower (from 15 to 50%, depending on times of dipping in water at 20°C after inoculation) (Figure 1). Similar results were observed when fruits were wounded and inoculated introducing 20 µl of a *M. fructicola* conidia suspension into the wound: all wounds of HT and control fruits resulted infected over the 80% in all assayed times (data not showed). With respect to the severity of disease, lesions diameters were significantly larger in heated fruits with respect to the controls when inoculation was performed in a short time after hot water treatment (until 6 hours) (Figure 2). Lesion diameter was for example 21.6% larger in treated fruits than in the controls when fruits were inoculated 1 hour after the hot water treatment.

4.3.2 Effect of heat treatment on M. fructicola *germ tube elongation on fruit surface*

The development of *M. fructicola* conidia on the surface of 'Red Haven' peach fruits treated with hot water and subsequently inoculated (immediately or after 24 hours) was microscopically observed. After 18 hours post inoculation, most conidia (over 95%) were germinated both in HT and control fruits (Figure 3). However, the conidia immediately (0 h) inoculated after heat treatment revealed elongation tubes significantly longer (135$\pm$7.8 µm) than that of conidia inoculated in control fruits (58$\pm$4.5µm) (Table 1). The stimulatory effect was absent when inoculation was performed 24 h after treatment. In this case no significant differences were found between germ tube length of conidia inoculated on heat treated (73$\pm$6.3 µm) and control fruit (72$\pm$4.8 µm) (Table 1).

4.3.3 Influence of VOCs emitted from heat treated peaches on **M. fructicola** *conidia germination*

Assays were carried out to evaluate the influence of volatile emission from hot water treated fruits on pathogen development, conidial cultures of *M. fructicola* were placed in the headspace surrounding treated fruits. Assays were performed on three cultivars of peaches to evaluate the influence of fruit volatile emission on pathogen development. Results showed that conidial cultures of *M. fructicola* exposed to the headspace of heated peaches formed more colonies than cultures exposed to headspace of control fruits (Table 2). The stimulation of conidial germination by volatile blend emitted from heated fruits ranged from the 64% in 'Springbelle' peach to the 33% in 'Lucie Tardibelle' peach.

4.3.4 Detection and identification of volatile organic compounds emitted by heated fruits using PTR-ToF-MS and SPME/GC-MS

The results on VOCs emission showed a significant difference between HT and control fruit.. On the whole, 50 compounds were detected by SPME/GC-MS in volatile blend of 'Lucie Tardibelle' peach (Table 3). The emission of some compounds was significantly higher in HT than untreated fruits: the aldehydes acetaldehyde, hexanal and nonanal, the alcohols 3-methyl-1- butanol, 3-hexen-1-ol (Z) and 1-octanol, the esters ethyl acetate, ethyl-3-methylbutanoate, isoamyl acetate and hexyl acetate, butanoic acid 3-methyl ethyl ester and 1-butanol 3 methyl acetate, and the alkan octane. Vice versa, the hydrocarbons alpha-pinene, toluene and pentadecane, and an unknown compound diminished or were not detected after the treatment. Ethanol was only detected in HT fruits, but its increase after treatment did not result to be statistically significant due to high variance. More insights into the behavior of ethanol emission upon heat treatment was provided by the PTR-ToF-MS measurements, and a clear and significant increase of both ethanol and acetaldehyde was found (Table 4). In fact, acetaldehyde and ethanol emission was fifteen and twenty eight folds higher in HT fruits compared to unheated ones, respectively. Moreover, PTR-ToF-MS measured VOCs not detected with SPME/GC-ME, such as methanol, which did not show any significant variation between HT and control fruits.

4.3.5 Effect of pure volatile organic compounds on **Monilinia fructicola** *growth*

Data on the effect of pure acetaldehyde and ethanol on *M. fructicola* growth revealed a stimulatory on the pathogen (Tab. 5). Pure acetaldehyde at the concentration of 0.6 µL L^{-1} induced an increase of conidia germination of 37.7%. In the same way ethanol vapours (0.2 µL L^{-1}) stimulated significantly the colony diameter with respect to the control (+15%) The colony

diameter of pathogen exposed to pure ethanol after 4 days at 25°C was of 39 mm while that of control was of 33 mm. No significant difference was reported in mycelia growth treated with the aldehyde.

4.4 Discussion

Heat treatment has been reported to be an effective method for the control of many postharvest diseases and generally it is used as curative treatment. In the present study we tested the preventive activity of hot water treatment to control brown rot on peaches. Our data showed for the first time that fruit heating few hours before pathogen inoculation could stimulate *M. fructicola* conidial germination . When inoculated in a short time (from 15 minutes to 6 hours) after dipping in water at 60°C for 60s, wounded and unwounded fruits showed in fact an incidence and severity of disease higher than control fruits (Fig. 1 and Fig. 2). These results are in contrast with effects of hot water treatment (dipping in water at 40°C for 5 or 10 min) applied before inoculation with *M. fructicola* observed by Liu et al. (2012) in a previous study, where heating was effective in reducing the lesion diameters for both times of treatment (5 and 10 min). These authors have also reported an increase of *chitinase* (CHI), *phenylalanine ammonia lyase*, and *β 1,3-glucanase* (GNS) gene expression on HT peach referring these changes to a possible role in host defense against fungal pathogen. In our trials the water temperature and the duration of treatment were respectively higher and lower than those used in the experiments performed by Liu et al. (2012) and this could explain the different response of fruits to treatment. In fact, in grapefruit treated by brushing at 62°C for 20 s an accumulation of CHI and GNS enzymes was correlated with an increase in resistance to *Penicillium digitatum* (Pavoncello et al., 2001).

In order to study the stimulation of *M. fructicola* on HT fruits, a microscope analysis of fruit epidermal tissues was performed. Prusky (1996) described that a spore pathogen once on the fruit skin, must attach to the surface, germinate, produce penetration structures or penetrate directly through wounds, and activate pathogenicity factors to develop pathogenicity process. Consistently, *M. fructicola* spores on HT and control fruits revealed a different behavior of germination depending on peach surface condition (Fig. 3). Indeed data showed that *M. fructicola* germ tube elongation was stimulated on HT peach surface (Table 1). The stimulatory effect became evident on peach heat treated and immediately inoculated with *M. fructicola*. After 18 h of incubation at 20°C, the pathogen germ tube length was 3 times longer on HT peach surface with respect to control fruits (Fig 3 a-b). Conversely, no differences were observed in germ tubes length of conidia on the skin of HT peach inoculated after 24 h from treatment and on the skin of untreated fruit (Figure 3c-d). This different behavior was supposed

to be related to volatile compounds emitted by fruits after hot water treatment and the results obtained in subsequent *in vivo* trials confirmed this hypothesis. A stimulatory effect of *M. fructicola* conidial germination was observed by the volatile blend emitted by heated fruits (64, 40 and 33% stimulation in 'Springbelle', 'Red Haven' and 'Lucie Tardibelle' peaches, respectively, (Table 2). Changes in components of the volatile emission consequent to hot water treatment were then detected by GC/MS and PTR-MS analysis, for the first time in literature. Most studies on peach volatiles in literature have focused in fact on changing during ripening and effects of aroma compounds on fruit flavor (Sumitani et al., 1994; Lavilla et al., 2002El Hadi et al., 2013). Data obtained from PTR-ToF-MS showed in particular a significant increase of acetaldehyde and ethanol in heat treated fruits compared to control ones. An increase in ethylene emission in heated fruits was also observed during the first hours after the treatment (data not shown). *In vitro* assays on *M. fructicola* with pure acetaldehyde (0.6 µL L^{-1}), and pure ethanol (0.2 µL L^{-1}) confirmed the specific stimulatory effect on conidial germination and mycelia growth, respectively. This is the first time that VOCs produced by heated peaches were tested on pathogen growth and identified. Few studies have been previously reported fungal stimulation by volatile organic compounds (Cruickshank and Wade, 1992; Eckert and Ratnayake, 1994; Filonow, 1999). However, our study confirms results of Cruickshank and Wade (1992) who found that ethanol and acetaldehyde produced during the ripening of apricots seemed to transform the mycelium of *M. fructicola* from latent into invasive, since these fruit volatiles were produced in parallel with symptom development of brown rot. In addition, Eckert and Ratnayake (1994) reported that a mixture of volatiles released by the wound and including ethanol and acetaldehyde stimulated conidia germination of *P. digitatum* and *P. italicum*. The effects of ethanol and acetaldehyde on fungal pathogens depends on concentrations of these compounds, since also fungal inhibition was also observed in literature after exposure to high concentrations of these volatiles (Avissar and Pesis, 1991; Gabler et al., 2005; Lurie et al, 2006). An increase of acetaldehyde and ethanol emission in the first hours following heat treatment was also found in mango fruits exposed to 48°C for 5 h (Mitcham and McDonald, 1993). It has been known that ethanol and acetaldehyde can be produced in senescent fruits and/or are associated with anaerobic processes in storage. For strawberry fruit stored in controlled atmosphere with 20% of CO_2 a primary cause of off-flavor appears to be related to the accumulation of volatile compounds such as acetaldehyde, ethyl acetate and ethanol. Our results shows that emission of acetaldehyde, ethanol and ethylene could be stress metabolites emitted in peaches as temporary effect of heat treatment.

In conclusion, results of our study showed that some volatiles emitted as temporary effect after hot water treatment could stimulate the germination of *M. fructicola* propagules. This

factor should be taken in consideration in order to a practical application of hot water treatment on fruit. This technique might require greater attention to the fruit management immediately after the heat treatment to avoid infection until 24 hours after treatment. Nevertheless hot water treatment remains a safety and friendly method to control brown rot before storage and shelf-life.

4.5 References

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4.6 Figures

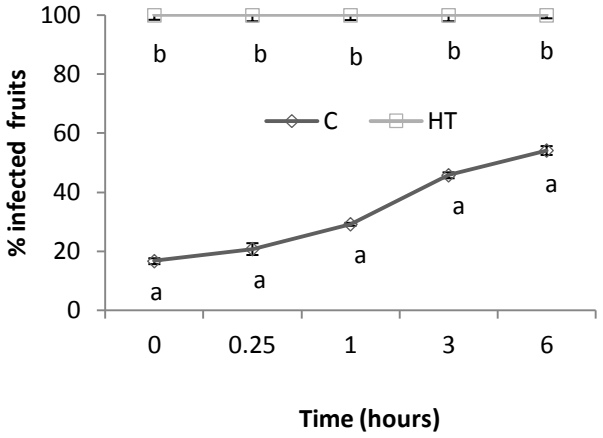

Figure 1 Effect of preventive hot water treatment on *Monilinia fructicola* percentage of infection (%) in unwounded 'Red Haven' peaches. Fruits were dipped in water at 60°C per 60 s. After 0, 0.25, 1, 3 and 6 hours after treatment, fruits were inoculated by immersion in *M. fructicola* conidial suspension ($4x10^3$ conidia mL^{-1}). Control fruits were dipped in water at 20°C for 60 s.The percentage of infected fruits was recorded after 5 days at 20°C. Within the same time, the same letters represent no significant differences according to LSD test ($P \leq 0.05$).

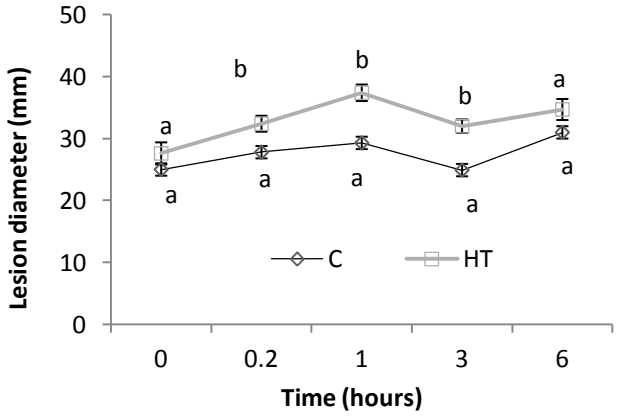

Figure 2 Effect of preventive hot water treatment on *Monilinia fructicola* lesion diameter (mm) in wounded 'Red Haven' peaches. Fruits were dipped in water at 60°C per 60 s. After 0, 0.25, 1, 3 and 6 hours after treatment, fruits were wounded and artificially inoculated with 20 µl of a *M. fructicola* conidial suspension (10^3 conidia mL^{-1}). Control fruits were treated by dipping in water at 20°C for 60 s. The lesion diameters were recorded after 5 days at 20°C. Each value is the mean of 24 fruits ± SE. Within the same time, the same letters represent no significant differences according to LSD test ($P \leq 0.05$).

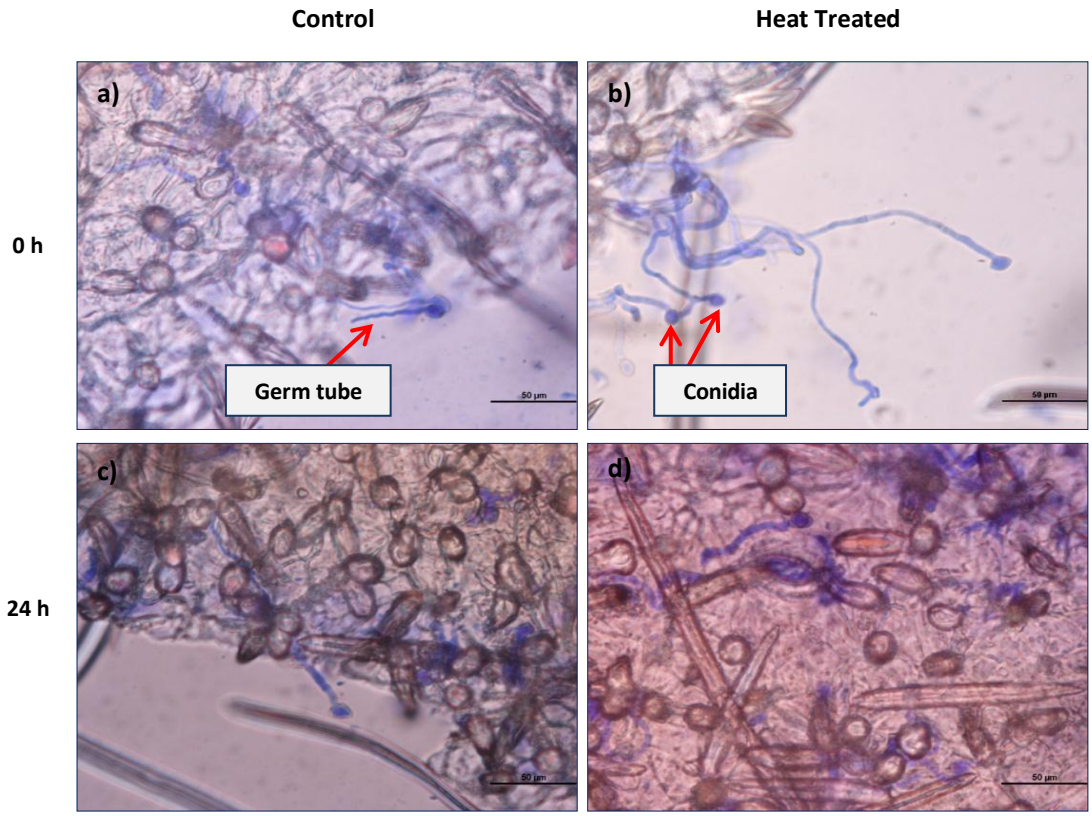

Figure 3 Pre-penetration events of *Monilinia fructicola* infection on peach fruit surfaces inoculated with a suspension of 10^6 conidia per mL. Conidia were stained with lactophenol blue and analysed under an optical microscope. (a) Control peach surface and (b) HT peach surface at 18 hpi (hours post-inoculation) with treatment and inoculation combined at time 0. Control peach surface (c) and (b) HT peach surface at 18 hpi with inoculation after 24 hours from the treatment. Bar = 50 µm.

4.7 Tables

Table 1

Influence of heat treated peach on germ tube length (µm) of *M. fructicola* conidia, inoculated on fruit surface. Fruits were treated by dipping in water at 60°C per 60 s and after 0 or 24 h were inoculated by spraying 5 mL for fruit of the pathogen conidia suspension (10^6 conidia per mL). Control fruits were represented by fruits dipped in water at room temperature. The length of conidia germ tubes was recorded after 18 h post inoculation at 20°C.

Time of inoculation from the treatment (h)	Control	Heat treated
0	58 ± 4.5 a*	135 ± 7.8 b
24	73 ± 6.3 a	72 ± 4.8 a

Each value is the mean of 30 replicates ± SE.
*Within the same row the same letter represents no significant difference according to LSD test ($P \leq 0.05$).

Table 2

Colony forming units of *Monilinia fructicola* spread on malt extract agar Petri dishes incubated at 25°C for 4 days. Each plate was overturned and placed on a mesh dividing from treated peach fruits inside the boxes.

Cultivar	**Control**	**HT**	**Stimulation (%)**
'Springbelle'	14.1 ± 1.3 b	39.0 ± 2.8 a*	64.1
'Redhaven'	12.4 ± 1.1 b	19.8 ± 1.8 a	40.0
'Lucie'	8.3 ± 0.8 b	12.0 ± 1.3 a	33.3

*Each value is the mean of 8 replicates ± SE. Within the same row the same letter represent no significant difference according to LSD test ($P \leq 0.05$).

Table 3

VOC emission of untreated and heated 'Lucie Tardibelle' peach as measured by SPME/GC-MS.

	CONTROL		HEAT TREATED							
Compound	MEAN	SD	MEAN	SD	*P-value*	p<0·05	Qual (%)	LRI	LRI Bibliography (CP WAX)	Formula
Acetaldehyde			1.7E+10	4.5E+09	0.003	*	78	709	700	C2H4O
Octane			4.3E+09	1.2E+09	0.003	*	91	802		C8H18
1,3-Butadiene,2,3-dimethyl + 1,3.Pentadiene, 4-methyl	2.8E+09	1.8E+09			0.057		74	806		
Unknown	9.1E+08	3.3E+08			0.009	*		821		C3H6O
Ethyl Acetate			1.4E+09	2.9E+08	0.001	*	91	900	882-890	C4H8O2
Ethanol			1.6E+10	1.2E+10	0.089		86	941	925-947	C2H6O
3-Heptene, 2,2,4,6,6 - pentamethyl	1.7E+10	1.3E+10	1.9E+10	4.7E+09	0.780		90	1001		C12H24
α-Pinene	6.9E+08	1.9E+08			0.003	*	87	1028		C10H16
Toluene	7.9E+08	4.1E+08			0.029	*	86	1053	1028-1049	C7H8
Butanoic acid, 3-methyl-, ethyl ester(Ethyl-3-methylbutanoate)	2.1E+09	2.1E+08	6.4E+09	2.1E+08	0.000	*	97	1084		C7H14O2
2,4,4,6,6,8,8-Heptamethyl-1-nonene	1.5E+09	1.5E+08	2.5E+09	8.6E+08	0.114		59	1097		C16H32
Hexanal	1.6E+09	4.0E+08	3.7E+09	7.1E+08	0.012	*	94	1100	1089	C6H12O
Unknown	2.7E+09	1.6E+09	3.4E+09	1.5E+09	0.600		64	1138		C7H14O2
1-Butanol- 3-methyl-acetate (Isoamyl acetate)			6.2E+08	2.5E+08	0.012	*	86	1187		C7H14O2
Heptanal	2.1E+08	1.3E+07	4.2E+08	2.3E+08	0.204		83	1198	1182-1188	C7H14O
D-Limonene	4.4E+08	1.8E+08	2.6E+08	1.9E+07	0.170		94	1207	1187	C10H16
3-Methyl-1-Butanol			1.3E+09	5.2E+08	0.012	*	88	1221		C5H12O
Furan-2-pentyl-	3.2E+08	2.8E+08	1.6E+08	2.8E+08	0.538		90	1244	1219-1225	C9H14O
1- Pentanol	2.4E+08	4.2E+08	7.4E+08	8.0E+08	0.396		64	1263	1253-1257	C5H12O
Acetic acid, hexyl ester(Hexyl acetate)	2.2E+09	7.6E+08	4.4E+09	9.5E+08	0.036	*	90	1284		C8H16O2
2-Butanone, 3-hydroxy- (Acetoin)	5.2E+08	9.1E+08	1.7E+09	9.5E+08	0.185		86	1297		C4H8O2
Octanal	9.2E+08	8.1E+08	1.3E+09	3.8E+08	0.460		78	1300	1284-1287	C8H16O
3-Hexen-1-ol, acetate (Z)	1.1E+10	4.5E+09	2.8E+10	1.0E+10	0.062		90	1329		C8H14O2
2-Hexen-1-ol,acetate, E-	3.3E+08	2.9E+08	1.2E+09	4.9E+08	0.062		78	1346		C8H14O2
6-Methyl-5-hepten-2-one	1.4E+09	1.4E+09	1.8E+09	7.2E+08	0.670		95	1349	1315-1332	C8H14O
1-Hexanol			3.6E+09	2.5E+09	0.069		90	1364	1357-1360	C6H14O
3-Hexen-1-ol (Z)			1.3E+09	6.6E+08	0.025	*	96	1394		C6H12O
Nonanal	3.9E+09	4.9E+08	1.3E+10	3.4E+09	0.009	*	95	1404	1384-1390	C9H18O
2-Hexen-1-ol (E)			1.5E+09	1.3E+09	0.130			1416		C6H12O
Pentadecane	3.0E+10	6.0E+09	1·9E+10	3.3E+09	0.046	*	97	1500		C15H32
Dodecanal or decanal	1.5E+09	2.5E+08	1.9E+09	3.7E+08	0.227		91	1508	1493 (decanal)	
Benzaldehyde	1.6E+09	8.0E+08	2.1E+09	1.1E+09	0.515		96	1533	1516-1518	C7H6O

Linalool	6.1E+10	8.3E+09	4.5E+10	9.0E+09	0.081		97	1556	1530	C10H18O
1-Octanol			1.1E+09	2.8E+08	0.002	*	86	1567	1562-1568	C8H18O
Hotrienol	1.1E+09	1.1E+08	1.0E+09	3.7E+08	0.695		86	1619		C10H16O
Acetophenone	4.8E+08	8.4E+08	1.3E+09	1.1E+09	0.385		91	1660	1652	C8H8O
1-Nonanol	7.2E+08	6.8E+07	2.1E+09	1.9E+09	0.287		91	1669	1667	C9H20O
Heptadecane	1.9E+10	9.6E+09	2.0E+10	3.5E+09	0.884		97	1700		C17H36
2(3H)-Furanone·5-ethyldihydro-(γ-Hexalactone)	1.8E+09	4.5E+08	2.5E+09	4.5E+08	0.159		91	1709		C6H10O2
5,9-Undecadien-2-one, 6,10-dimethyl- (Z) (cis-Geranylacetone)	4.9E+08	4.6E+08	7.0E+08	2.7E+08	0.538			1865		C13H22O
Octanoic acid,3-hexenyl ester (Z)(cis-3-hexenyl octanoate	7.7E+08	4.6E+08	6.5E+08	4.1E+08	0.738			1866		C14H26O
Nonadecane	8.9E+08	5.1E+08	1.2E+09	3.3E+08	0.437		96	1900		C19H40
Phenol, 2,6-bis (1,1 - dimethylethyl)-4-methyl-(Butylatedhydroxytoluene)	1.0E+10	5.1E+09	1.0E+10	4.0E+09	0.926		98	1923		C15H24O
Benzothiazole	1.0E+09	3.4E+08	7.1E+08	2.9E+08	0.305		87	1962	1961	C7H5NS
Hexadecanal			1.6E+09	1.3E+09	0.104		87		2109	C16H32O
6-Amyl,alphapyrone	3.2E+09	1.2E+09	1.9E+09	9.7E+08	0.218		95			C10H14O2
2H-Pyran-2-one, 6-heptyltetrahydro(δ-Dodecalactone)	1.3E+09	6.7E+08	7.4E+08	6.4E+08	0.330		90			C12H22O2

Significant differences ($P<0.05$) are marked with an asterisk. Reported values are expressed in chromatographic peak area units.

Table 4

Emission of selected VOCs from control and heated 'Lucie Tardibelle' peach as measured by PTR-ToF-MS.

		CONTROL		**HEAT TREATED**			
Ion sum formula	Annotation	MEAN (ppbv)	SD (ppbv)	MEAN (ppbv)	SD (ppbv)	P-value	*P<0.05*
CH5O+	Methanol	95.7	76.5	84.4	54.6	0.755	
C2H5O+	Acetaldehyde	27.5	22.4	445.5	294.7	0.003	*
C2H7O+	Ethanol	5.1	3.5	143.0	96.3	0.003	*
C2H5O2+	Acetate fragment	6.3	1.1	16.5	9.8	0.018	*
C10H17+	Monoterpenes	3.6	2.0	4.4	1.7	0.887	

Significant differences (*P<0.05*) are marked with an asterisk.

Table 5

Culturable conidia and mycelia growth *of Monilinia fructicola* treated with 0.6 ppm of pure acetaldehyde and 0.2 ppm of ethanol, respectively. The conidia were spread on Petri dishes and incubated at 25°C for 2 days, the diameter of mycelia was recovered after 5 days at 25 °C.

	Control	Acetaldehyde 0.6 ppm	Ethanol 0.2 ppm
CFUs	48.2 ± 6.2 **a**	77.3 ± 2.4 **b***	57.5 ± 6.4 **a**
Ø colony (mm)	33.6 ± 1.1 **a**	32.0 ± 3.2 **a**	39.0 ± 0.4 **b**

Each value is the mean of 9 replicates ± SE .

*Within the same row the same letters represent no significant differences according to LSD test (*P < 0.05*).

Transcriptional profiling of apple fruits in response to heat treatment: involvement of a defense response during *P. expansum* infection

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5.0 Abstract

In the last years, heat treatment seems to represent an effective and safe approach for control of postharvest fruit decay. In this study, the effect of hot water treatment (HWT) (45°C for 10 minutes 60°C) on blue mold of apple was investigated. In particular,HWTwas applied to "Ultima Gala" apples in two differentmannersi) apples were inoculated with *P. expansum* spore suspension and treated after 1, 4 and 24 hours (post-HT); ii) apples were treated at first and inoculated with *P. expansum*spore suspension after 1, 4 and 24 hours (pre-HT) and stored at 20°C for 6 days. Significant reduction in fruit rot incidence up to 100% was found in post-HT method at 4 and 24 hours while a 30% of reduction in blue mold incidence was found in HT apples at 1 and 4 hours (pre-HT). On the other hand, *in vitro* trialsshowedno lethal effect of HTfor 10 minutes at 45°C on *P. expansum*conidia suspension, suggesting a great resistance to heat of this pathogen. In order to investigate on the molecular mechanisms involved in fruit response upon heat treatment, a microarrayanalysis was carried outto provide a global transcriptional analysis of gene expression of apple upon 0, 15, 30 minutes, 1, 4, 8 and 24 hours from the heat treatment. Our microarray results showed further evidence that after 1 and 4 hours from heating, HT apples presented the highest number of differentially expressedgenes. Among the up-regulated genes,the large amount of induced *Heat Shock Proteins, Heat Shock Cognate Protein, Heat Shock Trascription Factors* genes, involved in acquired thermotolerance, support the idea that, after an heat shock, those genes required for the thermotolerance process, might be involved in the induced resistance response.

5.1 Introduction

Apples are a wide-spread crop, highly appreciated by consumers due to their sensory and nutritional characteristics (Giovanelli et al., 2014). Each country present diverse range of cultivated cultivars based on consumers choices. Apples are rarely consumed directly after harvest but prolonging the storage period from 6 to 12 months the world trade is ensured. Most rots of apples which appear during storage are caused by fungal infections already present on the fruit at harvest (Maxin et al., 2014). However, the restrictions in the use of fungicides in the European Union and other countries, the increase in organic production and consumer demand for fruit with residues much lower than levels established by regulations have stimulated research into alternative control measures. Furthermore, resistance development may impair the efficacy of fungicides against key pathogens (Weber and Palm, 2010) so alternative strategies to control fungal postharvest disease in order to limit high product losses are required. Among the physical treatments that exploit both direct effect on pathogens propagules and a condition to induce defence responses on fruit or vegetable products, the hot water treatment emerged as a completely safe technique based solely on the use of heat (Fallik, 2004). Heat treatments of apples have shown promise in reducing the subsequent development of storage rots (Fallik et al, 2001). As reported, Neri et al (2009) demonstrated the useful application of hot water treatment at 45°C for 10 minutes in reducing *Neofabraea alba* rots up to 90% of naturally infected fruit (cv Pink Lady) after 135 d of storage at 2°C.Maxin et al (2012a) have demonstrated significant reduction in incidence of rots on naturally infected apples by incubation period of 3 min at 50-54°C (dipping) and 20 or 25 s at 55°C (rising), followed by up to 100 d cold storage and 14 d of shelf life, respectively by 80-100% and 60-80%. Il was observed that heat treatment application on artificially infected fruit with *P. expansum* gave a significant discrepancy between the HT temperatures lethal to spore suspensions *in vitro* and those causing the inhibition of fruit rot development (Maxin et al., 2012b). The authors reported an initial suppression of fruit rots with a treatment at 47°C for 3 minutes while a direct inhibitory effect of heat of *P. expansum* in apples was evident only in pre-inoculated fruits exposed to hot water dipping at 70°C, whose, obviously, determined an heat scald effect. It has been noticed that many other effects are produced by heat treatment such as changes in epicuticular wax structures creating obstructions of deep cracks (Roy et al., 1994) with a follow diminishing calcium uptake (Lurie et al., 1996); a decrease in rate softening for “Anna”, non-storing early cultivar, and “Granny Smith”, long-storing late cultivarapples after 4 days at 38°C (Klein and Lurie, 1990) and, no less important, the activation of multiple protection mechanisms,a phenomenon called heat shock (HS) response (Charng et al., 2006). Sub-lethal heat treatments are known to have profound effects on the physiology of plant cells and tissues, including ripening fruit (Maxin et al 2012b). Plants

must cope with heat stress for survival, so they developed different mechanisms including the maintenance of cell membrane stability, capturing the reactive oxygen species (ROS), synthesis of antioxidants, accumulation and osmoregulation of osmoticum, induction of some kinases that respond to stress, Ca-dependent kinase proteins, and enhancing the transcription and signal transfer of chaperones (Wahid et al., 2007). In particular, among these, several classes of Heat Shock Proteins (HSP) are present in plants and include the small HSPs (sHSPs), ranging in molecular weight from 15 to 28 kD), HSP 60, HSP70 (and a constitutively expressed HS cognate protein, HSC70), HSP90 and HSP100 (Vierling, 1991; Gurley, 2000). As reported by Lee and Vierling (2000), sHsps prevent irreversible substrate aggregation bybinding heat-denatured substrates, and then present substrateto other cellular components for ATP-dependent refolding. The mechanism of sHsps action in conjunction with Hsp70 system is universal and is thought to occur in complexes ("refolding machines") containing HSC70/HSP70 and DnaJ homologes. With the progress of genome-wide gene expression studies employing technologies such as microarray analysis, a novel Hsp gene, named *Hsa32*, was isolated from tomato, (Liu et al, 2006). Consequently, homologous sequences of *Hsa32* were found in *Arabidopsis thaliana*, to be essential for acquired thermotolerance (Charng et al., 2006). In addition, an *Hsp101* was also determined as a key component to protect plants under heat stress (Gurley, 2000). Global analysis of mRNA expression has emerged as a valuable tool for elucidating gene expression in response to a wide range of biological processes such as disease resistance and susceptibility, environmental stress, and fruit development. This approach is particularly useful when applied to specific tissues and conditions wherein discovery of new genes can be efficiently pursued along with a global analysis of transcriptional profiles (Soria-Guerra et al., 2011; Vilanova et al., 2014). Understanding of molecular events associated with a heat treatment applied on apple fruit could provide valuable information for raising the popular acceptance of this method. This is the first time that a large-scale gene expression analysis was undertaken to analyze from 0 to 24 hours the molecular changes on apple fruit after a heat stress as the hot water treatment. In particular, the aim of this study was to highlight the differences in gene expression in "Ultima Gala" apples after heat treatment by using microarray analysis. Moreover, the effect of HT was also determined in *in vitro* trials on conidial germination of *P. expansum* and *in vivo* on artificially infected apples.

5.2 Materials and Methods

5.2.1 Fruit

The apple (*Malus domestica* Borkh.) cultivar 'Ultima Gala' was used in this study. Fruit samples were provided by the Appalachian Fruit Research Station, US Department of

Agriculture-Agricultural Research Service (USDA-ARS), located in Kearneysville (West Virginia, USA) and used immediately. Harvest was carried out on 9 August 2012. Fruits were selected foruniform size and without physical injuries or apparent infection. Flesh firmness, soluble solids and acidity were determined as quality parameters.

5.2.2 Quality parameters

Flesh firmness was measured on two opposite sides of each fruit with an 11-mm diameter plunger tip mounted on a drill press stand (Craftsman, Chicago, IL, USA). Total soluble solids content was assessed in juice using a refractometer (Atago, Tokyo, Japan) and titratable acidity by titration of 10 mL of juice with 0.1 N NaOH up to pH 8.2 and expressed as grams of malic acid per litre of juice. Data represent the mean of 15 fruits.

5.2.3 Fungal Cultures

P. expansum strain was obtained from USDA fungal collection. Petri dishes containing potato dextrose agar (Difco, Sparks, MD, USA) were inoculated with the isolate and incubated at 25°C for 7 days. Conidial suspension was prepared by adding 10 mL of sterile distillate water with 0.01% (w/v) Tween 80 by gently scraping the surface of the colony with a sterile spatula and passing the wash water through two layers of cheesecloth to remove hyphal fragments. Conidia concentration was adjusted to 10^4 conidia per mL using hemocytometer for the in vivo assay and 10^6 conidia per mL for the culturable conidia test.

5.2.4 Penicillium expansum culturable conidia test

Conidia viability was measured as colony forming units (CFU) on PDA (Casals et al., 2010). Aliquots of 0.5 mL spore suspension(10^6spores mL^{-1}) were added to 4.5 mL of water pre-warmed at45, 50, 55 and 60°C. Immediately after 5 and 10 min of exposure,0.5 mL of the warmed conidia suspension was diluted 100-fold incold water. Aliquots (0.1 mL) of treated *P. expansum*conidia suspensions were spread on Petri dishes andincubated for 3 days at 25°C. A suspension of untreated conidia (10^3conidia mL^{-1}) was used as the control. Approximately 100 sporesof each pathogen per treatment were evaluated by the culturableconidia test, expressing the results as number of CFU. The sampleunit was represented by four plates (replicate) and the experiment was conducted twice.

5.2.5 Infection trials of HT apple fruits with P. expansum

The application of an heat treatment for apple fruit was performed in two conditions: i) apples were artificially inoculated at time 0 and heat treated after 1, 4 and 24 hours (post Heat Treatment, post-HT); ii) apples were treated at time 0 and artificially inoculated after 1, 4 and 24 hours (pre Heat Treatment, pre-HT). Heat treated (HT) fruit were immersed in pre warm

water at 45°C for 10 min. Control (C) fruit were dipped for 10 min in water at 20°C (RT). For artificial inocula apples were wounded with a nail (1 mm wide and 2 mm deep) and infected with 20 µl of conidial suspension of *P. expansum* 10^4 mL^{-1}. Fruit were incubated at 20°C for 7 days. Each biological replicate consisted of five fruits for three replicates, the experiment was repeated twice. Data on pathogenicity and fruit quality parameters were analyzed for significant differences *(P<0.05)* by analysis of variance (ANOVA) using LSD test.

5.2.6 Fruit material for microarray and qRT-PCR analysis

'Ultima Gala' apples were harvest and immediately used. HT fruit were dipped in hot water at 45°C for 10 min, C fruit were treated by dipping in RT water for the same exposure time. In order to obtain a general overview of the mechanisms involved after the heat treatment on skin and mesocarp samples were collected using a peeler. Each biological replicate consisted in a pull of first and second apple layers (peel and pulp respectively) collected from three fruit sides from 9 apple fruits. Tissueswere sampled after 0 (immediately after the treatment), 15 and 30 minutes, 1, 4, 8 and 24 hours from the treatment. All samples were immediately frozen in liquid nitrogen, lyophilized for 7 days and ground to a fine powder for subsequent RNA extraction for the microarray analysis and qRT-PCR.

5.2.7 RNA Extraction

Total RNA was extracted as described by Vilanova et al., (2014). Briefly, 1 g of lyophilized apple tissue(pulp and peel) was added to a preheated mixture of 10 mLof extraction buffer (100 mM Tris‑HCl, pH 8.0, 2 M NaCl,25 mM EDTA, pH 8.0, 2 % (w/v) cetyltrimethylammonium,2 % (w/v) polyvinylpyrrolidone 40, 500 mg L^{-1} spermidine,2 % β-mercaptoethanol). After homogenization with aPolytron PT 10/55 (Kinematica AG, Lucerne, Switzerland)for 1 min, the extract was incubated at 65 °C for 15 min andcooled before 10 mL of chloroform‑isoamyl alcohol (24:1, v/v) was added. The homogenate was centrifuged at 10,000×gfor 10 min at 4 °C and the aqueous phase was re-extractedwith 10 mL of chloroform‑isoamyl alcohol (24:1, v/v) andcentrifuged at 10,000×g for 15 min at 4 °C. The supernatantwas transferred to different Eppendorf tube and nucleic acidswere precipitated overnight at 4 °C by adding a one thirdvolume of 7.5 M lithium chloride. After centrifugation at 12,000×g for 30 min at 4 °C, the pellet was washed in 70 %ethanol, resuspended in 80 µL of water, and pooled RNAfrom the same samples into a new Eppendorf. An equalvolume of phenol‑chloroform‑isoamyl alcohol (25:24:1, v/v/v) was added and the homogenate was centrifuged at 12,000×g for 5 min. The aqueous phase was re-extracted with ½ volume of chloroform‑isoamyl alcohol (24:1, v /v) andcentrifuged at 12,000×g for 5 min. The aqueous phase wasincubated with 1/10

volume sodium acetate and 2.5 volume95 % ethanol for 30 min at −20 °C and centrifuged at 12,000×g for 30 min. The resulting pellet was washed with 70%ethanol and centrifuged immediately at full speed for 1 mintwice. The pellet was dissolved in 200 μL of water. RNAconcentration was quantified using a ND-1000 Nanodropspectrophotometer (Thermo Scientific, DE, USA) and theintegrity was verified by agarose gel electrophoresis andstaining of the resulting gels with ethidium bromide.Extracted RNA was treated and purified with turbo DNAfreekit (Ambion, TX, USA) following the manufacturer'sinstructions. To remove DNAse products, extracted RNAwas incubated with 1/2 volume 7.5 M ammonium acetateand 3 volume 95 % ethanol for 1 h at −20 °C. Samples werethen centrifuged at 12,000×g for 30 min at 4 °C and finallydissolved in 200 μL of water.

5.2.8 Preparation of Labeled aRNA Probes

To prepare the fluorescent probes, 5 μg RNAwas amplifiedusing the Amino Allyl MessageAmp II aRNA AmplificationKit (Ambion, TX, USA) following the manufacturer'sinstructions. The resulting *in vitro* transcription products areconfigured to incorporate the modified nucleotide, 5-3(-aminoallyl)-UTP (aaUTP) into the aRNA (antisense amplifiedRNA) during in vitro transcription. aaUTP contains a reactiveprimary amino group on the C5 position of uracil that wascoupled to the N-hydroxysuccinimidyl ester-derivatizedreactive dyes, Alexa 555 and Alexa 647 (Molecular Probes,OR, USA), following the manufacturer's instructions. Beforeincorporation of the dyes, each sample was quantified using aND-1000 Nanodrop spectrophotometer (Thermo Scientific,DE, USA) and the overall quality verified by agarose gelelectrophoresis and staining of the resulting gels withethidium bromide.

5.2.9 Microarray Hybridization and Analysis

The experimental design was made as follows: each comparative hybridization between the two treatments consisted of three biological replicates and seven time points, including a dye-swap, for a total of 42 slides. All treatments weresubjected to a direct comparison with each other. LabeledRNA was hybridized on an apple microarray consisting of40,000 long-oligos (70-mer) printed on a single glass slide(Soria-Guerra et al. 2011). Slides were hydrated at 50 °C in a water bath and UV-crosslinked at 0.6 J cm^{-2} (Bioslink, NewHaven, CT, USA) before use. Slides were prehybridized inCoplin jars containing a solution of 20%formamide, 6× SSC,0.1 % SDS, and 5× Denhardt's solution, with 25 μg mL^{-1}tRNA during 45 min at 42 °C shaking at 60 rpm, sequentiallywashed with water 10 times, 20 times in ethanol, and thendried by centrifugation at 2,000×g for 30 s.Labeled aRNA probes, as indicated in Fig. 1, were mixeddried and resuspended in 60 μL of hybridization solution 1×(Ambion, Austin, TX, USA) and denatured for 5 min at 65 °Cin a heat block. Hybridizations were done at 42 °C for 24

husing an Arrayit hybridization cassette (Arrayit Corporation,Sunnyvale, CA, USA). Post-hybridization washes wereperformed in Coplin jars with 60 rpm agitation as follows:once in 1× SSC and 0.2 % SDS at 42 °C for 5 min, once in0.1× SSC, 0.2 % SDS at room temperature for 5 min, andtwice in 0.1× SSC at room temperature for 5 min. Finally,slides were dried by centrifugation at 2,000×g for 30 s.Microarray slides were then scanned with a GenePix 4000Bfluorescence reader (Axon Instruments Ind., Foster City, CA,USA) using GenePix Pro 3.0 image acquisition software (AxonInstruments Ind.).Within individual arrays (slides), spot intensitywas normalized using the GenePix software and nonhomogeneousor aberrant spots were discarded. Differentially expressed genes were identified using twointerconnected ANOVA models: the first model normalizedspot intensities across arrays (normalization model) and thenthe second model determined gene effects (gene model) (Wolfinger et al. 2001). Individual spot intensities, y_{ijk}, werefirst inputted to the normalization model:

$$y_{ijk} = \mu + A_i + T_i + (AT)_{ij} + \varepsilon_{ijk}$$

where μ represents an overall mean

A is the main effect for arrays

T is the main effect for treatments

AT is the interaction effect of arrays and treatments, and

ε is stochastic error.

The residuals derived from the normalization model for theindividual spots, r_{ijk}, were then inputted to the gene model:

$$r_{ijk} = \mu_k + S_{ik} + T_{jk} + \gamma_{ijk}$$

where S_{ik} is a main effect for spots and γ is stochastic error ($\mu$and T are described above). Analysis of both models wasperformed using the Mixed procedure in the SAS softwaresystem. (SAS Institute, Cary, NC. 2008). Differences in geneexpression were considered to be significant when the *P* valuewas less than 0.05.

5.2.10 RT-qPCR Analysis

In order to validate microarray analysis a qRT-PCR was performed. For the experiments, first-strand cDNA was synthesized from 1 μg of total RNA in 20μl reaction volume using ImPromII (Promega), following the manufacturer's instructions. Primers were designed using the online program Primer3. The sequence of each primers with annealing temperatures used in

this work were listed in Table 1. Amplifications were run in a 96 well-plates MX3000 thermal cycler (Stratagene, La Jolla, CA, USA). In a total volume of 12.5 µl, each reaction mixture contained1X of Platinum Sybr- Green Master mix (Invitrogen, Milan, Italy), 0.5 µL of each primer (5 µM), 3.25 µL of nuclease-free water and 2.5 µL of 1:12.5 of cDNA. The following cycling conditions were used: an initial denaturation step at 10 min at 95°C, followed by 40 cycles consisting of 30 s at 94°C, an annealing step at a specific temperature (Table 1) and an extension step at 72°C for 1 min. Fluorescence was monitored at the end of each annealing step. To assess the amplification specificity, melting curve analysis was always performed at the end of each experiment, by monitoring the fluorescence from 55 °C to 95°C, every 0.1 °C. Quantification was carried out using the standard curve method (Applied Biosystem 2001). Standard curves were generated by serial dilutions of a randomly chosen cDNA first strand. Data were analyzed using MXPro QPCR Software version 3.0 (Stratagene, USA). For each sample, three independent biological replicates were made and each replicate was run twice. The expression of each gene was normalized to the amount of actin housekeeping gene. Means from independent experiments were subjected to one-way ANOVA.

5.3 Results

5.3.1 Influence of hot water treatment on P. expansum conidia germination

Conidia germination of *P. expansum* was almost completely inhibited after a dipping in hot water at 55°C for 10 min (Fig. 1). *P. expansum* conidia viability (number of CFU) was inhibited by 77% reducing the exposure to 55° at 5 min. The temperature 50°C showed a lethal effect after 10 minutes with a 31% of killed conidia compared to the control (20°C) while no significant differences were shown, for the same temperature, reducing the exposure time to 5 min. The application of an heat treatment at 45°C for 5 or 10 minutes had no lethal effect on the conidia viability of *P. expansum*.

5.3.2 Influence of hot water treatment on apple fruit artificially inoculated with P. expansum

Two different methods of heat treatment were employed: : i) apples were artificially inoculated at time 0 and treated after 1, 4 and 24 hours (post-HT); ii) apples were treated at time 0 and artificially inoculated after 1, 4 and 24 hours (pre-HT). In both two experimental treatments, HT (45°C for 10 min) showed a great rot control in terms of disease incidence (Fig. 2 a-b). Among the assays the method post-HT (ii) where fruit were inoculated and subsequently heated gave the best control level reaching the 100% of rot reduction if the apples were heat treated after 4 and 24 hours from the inoculum (Fig. 2a) post-HT showed no effect at time 1 h. On the other hand the pre-HT where fruit were treated and inoculated after 1, 4 and 24 hours

has shown a rot reduction of 30% in HT apples compared to the control at time 1 and 4 (Fig. 2b), while no differences were reported at 24 hours. No visual symptoms of heat damage were observed in fruit treated with hot water for 10 min at 45°C. Flesh firmness was about 10,0 ±1,8 Kg, soluble solids content was 14,1 ±1,01 and 3,5 grams per liter of malic acid was also detected at harvest. No off-flavors or anomalous softening were observed by sensory evaluation in fruit treated, nor were significant differences found in any cultivar for sweetness, acidity, fruitiness, juiciness and hardness in treated fruit in comparison to the control (data not reported).

5.3.3 Microarray analysis

In order to investigate on the molecular mechanisms involved in fruit response upon heat treatment, a microarrayanalysis was carried out to compare gene expression of HT apple fruit dipped in warm water at 45°C for 10 min and control fruit treated at 20 °C following the same conditions.In particular, seven time points were chosen after the treatment to analyze the trend of gene expression from time 0 to time 24 hours in HT and Control fruit. From the comparison between the transcriptprofiling of these two conditions during the time course, nine datasets were generated:i) transcripts differentially expressed ($P < 0.05$) between HT and Control fruit at time 0, 15, 30 minutes, 1, 4, 8, 24 hours from the treatment are reported in Tables 2-8; ii) transcripts differentially expressed ($P < 0.05$) between HT and Control fruits commonly regulated on two time points (30 minutes and 1 hour) (Table 9); iii) transcripts differentially expressed ($P < 0.05$) between HT and Control fruits commonly regulated on four time points: 15, 30 minutes, 1 and 4 hours from the treatment (Table 10).The heat map representing a comparison of the heat treated and control samples for the seven time points, shows that the major set of genes were found up-regulated in particular from 15 minutes to 4 hours in HT apples (Fig. 3). To confirm the differences in gene expression and calculatetranscript levels, 9 genes found differentially expressed in HT fruit compared to Control ones after 30 minutes and 1 hour from the treatment wereassayed by qRT-PCR (Fig. 4 and Fig. 5).

5.3.4 Transcripts differentially expressed between HT and Control fruit at time 0

At time 0, immediately after the treatment, 221 genes were found to be differentially expressed ($P<0.05$) in the HT apples compared to the control. In particular, 140 genes were induced in HT fruit and 15 of them showed an higher expression level than 1.7-fold compared to the control. Moreover, 81 genes showed a lower significant expression level in treated fruit compared to the control, the values of 3 genes were found to decrease 2-fold in HT apples. The data showed in Table 2 reported GO codes and Go terms for each probe differentially expressed with a $P < 0.05$. Among the induced genes *18.5 KDa heat shock protein*, involved in stress

response functions, and an *LRR receptor-like serine/threonine-protein kinase EFR* noted to be involved in the detection of external stimuli and defense response were found to increase immediately (30 seconds maximum) after the treatment.

5.3.5 Transcripts differentially expressed between HT and Control fruit at time 15

Microarray results reported an increase in number of genes differentially expressed after 15 minutes from the treatment. In particular, a total of 225 genes were found to be significantly influenced by the treatment. As reported in Table 3,a total of 113 genes were induced and 112 suppressed. Among them, 60 genes were found to be upregulated more than 1.7-fold higher in HT samples compared to the Control, while 11 genes were suppressed of 2 fold in HT fruit with respect to non treated ones. Focusing on the results, after 15 minutes genes encoding for *Probable pyridoxal biosynthesis protein PDX1.2* (MDP0000543224),*Heat shock cognate 70 kDa protein 4* (MDP0000684170),*18.5 kDa class I heat shock protein*(MDP0000207407), *Heat shock protein 101* (MDP0000303015), *17.5 kDa class I heat shock protein*(MDP0000574524), *Heat shock protein 70KDa* (MDP0000311339) were found to be induced by the heat treatment with a ratio 3.3, 2.9, 2.4, 2.3, 2.3 and 2.3, respectively, comparing to the control fruits. Two genes encoding for *Glycogenin-1* (MDP0000466683 and MDP0000446914), and a*Probable galacturonosyltransferase-like 10*(MDP0000120125), involved in carbohydrates biosynthesis increase their expression level. On the basis of GO classification, among the up-regulated genes at time 15 minutes from the treatment, 37 resulted involved in defense response increased by heat abiotic stress. Among the 11 genes showing 2- fold changes lower in HT fruit compared to the control ones, an *auxin-repressed 12.5 kDa protein* (MDP0000323212) involved in signal transduction has been reported. Moreover a*WRKY transcription factor 19*(MDP0000551283) belonging to the large family of plant transcription factors involved in the regulation of various abiotic and biotic stresses was also found down-regulated.

5.3.6 Transcripts differentially expressed between HT and Control fruit at time 30

A total of 575 genes resulted differentially expressed ($P< 0.05$) after 30 minutes from the heat treatment (45°C 10 min) in HT samples compared to the control. Among these, 362 were found induced (93 genes with a ratio > 1.7) and 212 suppressed (11 with a ratio <0.5). The Table 4 reported the list of genes differentially expressed showing a ratio value more than 1.7 for the up-regulated genes and lower than 0.5 for the down-regulated ones. The most up-regulated gene was found a *conserved oligomeric Golgi complex subunit*, 3.3-fold higher in HT samples compared to the control. Among genes involved in stress response, two genes encoding for a *heat shock cognate 70 KDa protein 4* (MDP0000684170 and MDP0000122734), a *heat shock protein STI*, (MDP0000161691), a *Heat shock 70 kDa protein* (MDP0000311339), and a

protein popC (MDP0000744777) showed a ratio higher than 2.0. Genes involved in cell proliferation are also induced such as *EXO*(MDP0000158045), *cyclin-D3-2*(MDP0000440811) and *conserved oligomeric Golgi complex subunit* 7 (MDP0000150261). After 30 minutes from the treatment, HT samples showed an *enolase* (MDP0000939989) down-regulated of 2.5-fold lower compared to the control, while genes encoding for *ethylene-responsive transcription factor 5*, *auxin-repressed 12.5 kDa protein*, and a *flavonol synthase/flavanone 3-hydroxylase* were found down-regulated of 2-fold in HT apples respect the control fruit.

5.3.7 Transcripts differentially expressed between HT and Control fruit at time 1 hour

The expression profile of genes (P< 0.05) in HT samples compared to the control after 1 hour from the heat treatment (45°C 10 min) is shown in Table 5. A total of 257genes were found up-regulated, of these 152 genes showed a ratio value higher than 1.7. On the contrary, 319 probes were found to decrease their expression levels on apple after the treatment and 34 of which resulted to be down-regulated with a ratio value lower than 0.5. The most up-regulated gene was *putative pyridoxal biosynthesis protein PDX1.2* (MDP0000543224) that reaches the highest ratio value (4.8) at 1 hour from the treatment. *Conserved oligomeric Golgi complex subunit 7* showed a ratio value 3.7. Genes encoding for proteins linked to stress response with a ratio value higher than 2.0 were *Heat shock cognate 70 kDa protein 4* (MDP0000684170), *Heat shock 70 kDa protein* (MDP0000311339), several *heat shock proteins as* two *18.5 kDa class I heat shock proteins* (MDP0000164489 and MDP0000207407) *and 17.3 kDa class I heat shock protein*(MDP0000247652),*Small heat shock protein, chloroplastic* (MDP0000125300), genes encoding for 1-*aminocyclopropane-1-carboxylate oxidase homolog 1* (MDP0000878773)and (PPPDE) *putativepeptidase domain contain protein* (MDP0000849143). Genes involved in carbohydrate biosynthesis process, such as *Glycogenin-1* (MDP0000466683 and MDP0000446914), *Isoamylase-1* (MDP0000313388) and *Triosephosphate isomerase*, (MDP0000152242) were found induced with a ratio value higher than 2.0. Among the 34 down-regulated genes *Squalene monooxygenase* (MDP0000266638), involved in the brassinosteroid pathway showed to be repressed 2.5-fold in HT apple compared the control. Both the *Indole-3-acetic acid-induced protein ARG7* gene, involved in the defense response and a gene encondig for the *β*-glucosidase 12 (MDP0000047589) were found down-regulated of 2-fold in HT apples compare to the control.

5.3.8 Transcripts differentially expressed between HT and Control fruit at time 4 hours

A total of 356 genes resulted differentially expressed (*P< 0.05*)in HT samples compared to the control after 4 hours from the heat treatment (45°C 10 min), of these 288 were found to be induced and 68 suppressed.The genes differentially expressed showing a ratio value more

than 1.7 (119) and lower than 0.5 (2) are listed in Table 6. The most suppressed gene was a *Probable serine/threonine-protein kinase* (MDP0000261706) whose expression level was found to be 3,3-fold lower in HT samples compared to Control fruit. On the other hand, the most up-regulated gene is a *Somatic embryogenesis receptor kinase 1* (MDP0000302297) encoding for protein belonging to the phosphotransferase family. The involvement of several heat shock proteins, as reported for the previous time points analyzed, were also shown in the Table 6. For instance gene encoding for, *17.9 kDa class II heat shock protein* (MDP0000291831), *Heat shock 70 kDa protein* (MDP0000311339) and a *Heat shock protein STI* (MDP0000161691) were found to be induced. A gene encoding for a *Glycogenin-1*(MDP0000466683 and MDP0000446914), showed an higher expression level on HT fruits compared to the control ones. Besides *HSPs*, several other genes involved in heat acclimation process such as *1-aminocyclopropane-1-carboxylate oxidase homolog 1* (MDP0000878773),*70 kDa peptidyl-prolyl isomerase* (MDP0000141863), an *unknown protein* (MDP0000508644), a *oxidoreductase* (MDP0000549793), and *transcription factors* (TFs) were found differentially expressed in HT samples after 4 hours with respect to the control ones.

5.3.9 Transcripts differentially expressed between HT and Control fruit at time 8 hours

A total of 148 genes were found differentially expressed (*P< 0.05*) at time 8 hoursfrom the heat treatment (45°C 10 min), of these, 105 were induced by the heat treatment and 43 down-regulated(Table 7). The most up-regulated gene was an unknown protein (MDP0000209621). Two transcription factors such as a *WRKY 11* (MDP0000946614) and *Transcription factor bHLH62* (MDP0000205113) showed a ratio value of 2.0 and 1.7 respectively.. A *Dihydroflavonol-4-reductase* (MDP0000322755), involved in flovonoid synthesis pathway was also found up-regulated of 1.8 fold. Among the suppressed genes, only those involved in cellular processes, such as *Patellin-5* (MDP0000145780), required in vesicle traffickingevents, were found. At time 8 hours a reduced number of differentially expressed genes was observed.

5.3.10 Transcripts differentially expressed between HT and Control fruit at time 24 hours

A total of 364 genes were found differentially expressed (*P< 0.05*) in HT fruit compared to the control ones at time 24 hours from the heat treatment, (45°C 10 min), 235 of which were up-regulated and 129 down-regulated. Among these, only seven genes showed a ratio value more than 1.7 and no genes with a ratio value lower than 0.5 has been found Table (8). In particular, two *17.9 kDa class II heat shock protein* (MDP0000291831 and MDP0000700383), a *18.5 kDa class I heat shock protein* (MDP0000207407) together with a *glutathione S-*

transferase (MDP0000755113) gene, with a ratio value of 2.0, 1.9 and 1.9 respectively were found up-regulated.

5.3.11 Transcripts commonly regulated after 30 minutes and 1 hour in HT and Control fruits, and qRT-PCR analysis

A total of 179 genes were found to be commonly regulated ($p<0.05$) at 30 minutes and 1 hour upon heat treatment as reported in Table 9. Of these126 were up-regulated and 53 down-regulated in common among two time points considered. In order to validate the microarray analysis, six up regulated genes and four down regulated genes were tested by qRT-PCR. A constant trend of up-regulation with a similar ratio was confirmed by qRT-PCR for *Heat shock protein STI* (MDP0000161691), *18.5 kDa class I heat shock protein* (MDP0000207407), *Granule-bound starch synthase 2* (MDP0000842179) involved in starch synthase pathway.*aminocyclopropane-1-carboxylate oxidase homolog 1* (MDP0000878773) involved in ethylene biosynthesis and *Glycogenin-1* (MDP0000466683) required in carbohydrates metabolism (Table 9, Fig 4). The *Aspartate-semialdehyde dehydrogenase* (MDP0000220526) gene showing down-regulation at 30 minutes and up-regulation at 1 hour from the treatment in microarray analysis, revealed the same trend by qRT-PCR (Table 9; Fig. 4). Similarly, the three down-regulated genes *Homeobox-leucine zipper protein HAT14* (MDP0000135540),*Tyrosine aminotransferase*(MDP0000180890) and *Phenylalanine ammonia-lyase 1*(MDP0000388769) was found to decrease in HT samples also by qRT-PCR analysis (Table 9; Fig. 5).

5.3.12 Transcripts commonly regulated in HT and Control fruit at 15 and 30 minutes, 1 and 4 hours

The expression profile of genes in common for four times points (15 and 30 minutes, 1 and 4 hours) after the heat shock, was shown on Table 10. A number of 33 listed probes were found differentially expressed ($P< 0.05$) in HT samples compared to the control. *Probable pyridoxal biosynthesis protein PDX1*.2 presented the higher ratio value at time 1 hour, maintaining the up regulation also for the other time points. Among these genes two *Heat shock cognate 70 kDa protein 4* (MDP0000684170 and MDP0000122734), and five *HSPs,* such as *18.5 kDa class I heat shock protein* (MDP0000207407), *Heat shock protein 101* (MDP0000303015), *Heat shock 70 kDa protein*(MDP0000311339)*17.5 kDa class I heat shock protein* (MDP0000574524) and *Heat shock protein STI* (MDP0000161691) were found activated in response to heat stress for all the time points analyzed. Moreover, 2 genes enconding for *Glycigenin-1* (MDP0000466683MDP0000446914), involved in carbohydrate metabolism resulted to be induced from 15 minutes to 4 hours from the heat treatment on apple fruits.

5.4 Discussion

The increasing interest on safe methods for the control of postharvest diseases due to focus on the heat treatments as promise approaches. Physical treatments, such as the use of heat, have been considering by many authors as alternative methods to the control of apple rots such as *P. expansum, Neofabrea. alba*(Neri et al., 2009; Maxin et al., 2012; Maxin et al., 2014) or brown rot of peach and nectarine fruit (Bustamante et al., 2012; Spadoni et al., 2013) and other fruits such as tropical banana and papaya fruit (Wang et al., 2012; Chávez-Sánchez et al., 2013). Among the causal agents of many postharvest fruit rots, *Penicillium expansum* is one of the most important fungal pathogens of apple in storages in all producing country (Vilanova et al., 2014). Conidia of *P. expansum* are the major type of inoculum responsible for fruit infection in fact they are presentin orchards, fruit storage rooms, dump-tank andflotation-tank water, and postharvest drencher andpacking facilities. *P. expansum* invades apple fruitprimarily through wounds such as punctures andbruises that occur at harvest or during postharvesthandling (Rosenberger 1990;Yin et al., 2013). Moreover is not recent the characterization of *P. expansum* isolates fungicide-resistant in USA (Kinay et al., 2007) and Europe (Mari et al., 2003). In the present work the hot water immersion at 45°C for 10 minutes of "Ultima Gala" apples was tested for the control of *P. expansum* in artificially infected fruit. In parallel, the molecular mechanisms of heat treatment were investigated using a transcriptomic approach, with the aim to focus on the heat treatment as an alternative method to apply during postharvest handlings. In particular, microarray analysis was used to analyze the differences on gene expression involved on HT apple fruit response during the first 24 hours after heating (Fig. 3). In order to clarify the mechanism of action of the heat treatment on blue mold, the inoculum of *P. expansum* was made on pre-HT and post-HT conditions. A complete inhibition of fruit rots, as reported in Fig. 2a (post-HT), was found after 4 and 24 hours from the HT on artificially infected apples. This is in accordance with Maxin et al (2012) who have reported 52-82% of suppression of blue mold infections dipping artificially infected fruit for 3 minutes at temperature ranging between 47-52°C. On the contrary, no results in percentage of reduced rots was found between HT and control fruit if the treatment was applied after 1 hour on previously artificially infected fruits (Fig. 2a). This may be explained focusing on the effect of the heat treatment on *P. expansum* conidia germination. As reported on Fig 1,*P. expansum*conidia showed a great resistance to heating compared to other pathogen such as *Monilinia* spp as reported by Spadoni et al.(2013). The authors, in a recent work, demonstrated that *M. laxa, M. fructigena* and *M.fructicola* conidia germination were completely inhibited after exposure of 60 s at 55°C. In the case of *P. expansum*, a significant reduction in conidial germination of 75% was detected only after the exposure at 55°C for 5 min. Increasing the exposure time at 55°C up

to 10 minutes the conidia germination of the pathogen was reduced more than 90% but not completely killed (Fig.1). Maxin et al., (2012) found a direct inhibitory effect of heat on *P. expansum* in apples only in pre-inoculated fruit exposed to hot water dipping at 70°C, with consecutive negative effects on fruit physiology (heat scald). The exposure conditions used for our*in vivo* trials (45°C 10 min) had no effects on pathogen viability, as non-germinated conidia, after 1 hour from the treatment (Fig. 2a). We can assume that *P. expansum* germinated conidia (after 4 and 24 hours from the inoculum) were more susceptible to heat compared to ungerminated condia. Obviously very short treatments (10–30 s) would be optimal to accelerate fruit handling in packing houses and would be better for commercial application than 45°C for 10 min (Neri et al., 2009). However, as demonstrated for *N. alba*a temperature of at least 50°C was needed to significantly reduce mycelia growth for short exposure treatment (1 min) but negative effects were visible on apples dipped at 50–55 °C for 30–180 s that showed skin damage, with sensitivity varying among cultivars (Maxin et al., 2005; Neri et al., 2009). In order to demonstrated the involvement of heat stress induced mechanisms on apples fruit, in accordance with Maxin et al. (2012) who proved that apples were resistant to *P. expansum* rot even if fruit were wound-inoculated with this fungus *after* the heat treatment, *in vivo* trials showing the pre-HT application results were carried out. As reported in Fig. 2b a 30% of significant reduction in pre-HT infected fruit at 1 and 4 hour from the treatment suggested the possible role of ripe apple defense response after an heat shock. In general, abiotic or biotic stress agents induce to modify signaling pathways. Our microarray data, validated by qRT-PCR on nine differentially expressed genes, demonstrated the predominant role of HSPs, increasing in number and type, during all time points analyzed, from 0 till 24 hours. It was evident that a minor number of genes were suppressed in HT apple compared to the number of induced genes. The highest number of down-regulated genes were found at time 1 hour from the treatment in HT apples. None of them showed a similar suppression level for consecutive four time points, as reported for the up-regulated genes (Tab. 10). At time 1 hour after the treatment (Table 5), 9 genes resulted to be more than twofold suppressed in heat treated fruit, among them we reported genes encoding for TFs, a kinase protein, TIFY proteins noted to be repressed by jasmonate responses and a *Mannan endo-1,4-beta-mannosidase 6*, encoding for a plant enzyme acting on N-glycan. Considering the molecular level of the genes in common in time points 30 minutes and 1 hour (Tab. 9), *Phenylalanine ammonia-lyase 1* (MDP0000388769, MDP0000139075), encoding for PAL, the key enzyme of flavonoid biosynthetic pathway (Flores et al., 2014) was found to decrease in HT apples. This is in contrast with Liu et al. (2012) for HT peach fruit where an increase in Chitinase, β-1,3-glucanase, and phenylalanine ammonia lyase gene expression and enzyme activity was demonstrated after an hot water for 5

and 10 minutes at 40°C and correlated to a reduction in peach brown rot lesion diameter. In our recent work on heat treated peach fruit we found a significantly up-regulation of *PAL* only after 15 minutes from heat treatment (Spadoni et al., 2014). Similarly to *Pal*, the *Flavonol synthase/flavanone 3-hydroxylase* (MDP0000906115)gene, involved in flavonoid biosynthesis (Flores et al., 2013), was also found down-regulated, suggesting a general suppression of flavonoid pathway upon heat treatment. Also *Tyrosine aminotransferase* (MDP0000180890), which converts tyrosine to hydroxyphenylpyruvate was suppressed in HT apples and this is in accordance with Fletcher et al., (2005) that found heat stress significantly reduced levels of total phenolic acids (71–87%) and soluble phenols(75–87%) in heat-stressed plants of *Mentha spicata L*.Studies on *β-Glucosidases* (β-glucoside glucohydrolase, EC 3.2.1.21) that catalyzes the hydrolysis of β-D-glucosides, have reported its role in different key metabolic events including the release of flavor volatiles (Hosel, 1981). Besides, the down-regulated *S-linalool synthase* (MDP0000192151), also implicated in scent production in flowers of *Clarkia breweri* (Dudareva et al., 1996), might slightly reduce aroma fruit perception also in apples.In addition, any kind of stress, it has been observed by many authors to influence the development of HSPs. Recently HSPs have been considered as biochemical markers for postharvest chilling stress in fruits and vegetables (Aghdam et al., 2013) or, as reported in the pulp of ripe banana, uponwounding (Chen et al., 2009). Also the specific proteins responsible for regulation of the expression of genes encoding HSPs, known as heat shock transcription factors (HSTFs), exert a protective action against stress. HSTFs function as direct sensors of H_2O_2 in plants so they not only have a role in regulating HSPs gene expression but they also take part in the regulation of the expression of genes encoding for antioxidant proteins and are thus able to raise resistance against oxidative stress (Panchuk et al., 2002). In our case, at time 0 (Tab. 2) only one small HSPs, the *18.5 kDa class I hsp* (MDP0000323296), was up-regulated twofold in HT fruit compared to control ones. From 15 min to 4 hours, induced genes small HSPssuch as, *18.5 kDa class I hsp* (MDP0000323296,MDP0000207407), *17.5 kDa class I hsp* (MDP0000574524), *17.9 kDa class II hsp* (MDP0000700383) in association with *Hsp 70 kDa*(MDP0000311339) and *Heat shock cognate 70 kDa protein 4* (MDP0000684170) and*DnaJ homologs* (MDP0000290546,MDP000079515) confirmed what reported by Lee and Vierling (2000) (Tab.3 and Tab. 9). The authors, using a heat-denatured firefly luciferase (Luc) bound to pea (*Pisum sativum*) Hsp 18.1 as a model to define the minimum chaperone system required for refolding of a Hsp-bound substrate, have demonstrated a refolding cooperation among sHsp with Hsc/Hsp 70 kDa proteins plus DnaJ homologs. In addition, *Hsp 101* (MDP0000303015), as reported in Tab. 10 to be up-regulated in HT fruit from 15 min to 4 hour after the treatment, it has been demonstrated to be required for a thermotolerance adaptation. Queitsch et al. (2000),

exposing *Arabidopsis* seedlings to a preconditioning at 38°C followed by a lethal temperature of 45°C for 2 h, showed an acquired thermotolerance in vegetal tissues with induced level of Hsp101 and consequently survival of plants. From our experiment other Hsps were induced with similar trend during the four time points analyzed. *Hsp STI* (MDP0000161691) defined as 'stress-inducible protein', up-regulated in HT apple fruit was also indicated in *A. stolonifera*, to may contribute to wholeplantthermotolerance in perennial grasses (Xu et al., 2011). *Hsa32*, encoding for a 32kDa protein, induced in apple fruit after 15 minutes from the treatment till 4 hours and found the homolog sequences in *A. thaliana* (AT4G21320), was reported to be one of the top candidate targets of the key heat shock transcription factors in *Arabidopsis* (Charng, et al. 2006). The authors discovered that *Hsa32* is not required for the normal growth and development of *Arabidopsis* but transcriptional and translational regulation of *Hsa32* by heat stress suggested that it is required for thermotolerance in plants (Charng et al., 2006). The reduction in number of differentially expressed genes after 8 and 24 hours (Tab. 6-7) upon the hot water immersion lead to consider this shock, to which apples are subjected, as a temporary effect. As described above, pathogens such as *P. expansum*, showing a great heat tolerance, it was expected to be able to grew on apple tissue treated by HT. Conversely it was observed a reduced rot incidence in pre-HT after 1 and 4 hours (Fig. 2b). In support of acquired resistance hypothesis, ourmicroarray resultsshowed a number of genessuch as Hsps, Hscs, HSTFs, etc increasing their expression levels in HT apples compared to Control ones, from 15 minutes to 4 hours from the treatment. Those genes are particularly known to be involved in acquired thermotolerance process. In conclusion the present study provides a comprehensive identification and characterization of gene expression in apple tissue in response to heat treatment. Our data confirm and extend the hypothesis that the response of HT apples involves the up-regulation of genes implied in thermotolerance. The higher induction of several genes involved in this metabolism was found in HT apple tissue collected after 1 and 4 hours from the heat treatment as noticed on heat map (Fig. 3). Results from pre-HT experiments showed a 30% of blue mold reduction without effect *P. expansum* viability, as suggested previously. These aspects, taken together, support the idea that, after an heat shock, genes requested to protect cells in acquired thermotolerance process might be involved in the induced resistance response. From our knowledge this is the first study on apple fruit examining the global changes in gene expression on HT fruit for seven time points (within a day) after the heat treatment.

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5.6 Figures

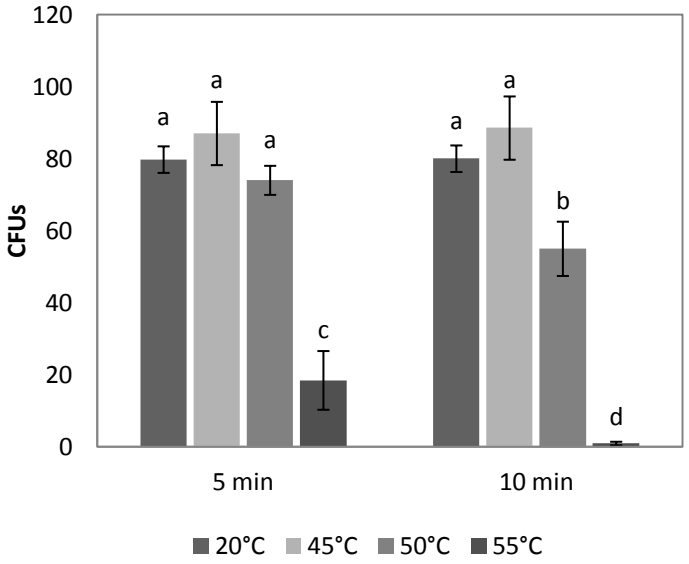


Figure 1 Culturable conidia of *Penicillium expaunsum* treated in a water bath at 20°C (control) and at 45°C, 50°C and 55°C for 5 and 10 min. The conidia were spread on Petri dishes and incubated at 25°C for 3 days. Each value is the mean of four replicates ± standard error. Within the same species the same letters represent no significant differences according LSD test ($P < 0.05$).

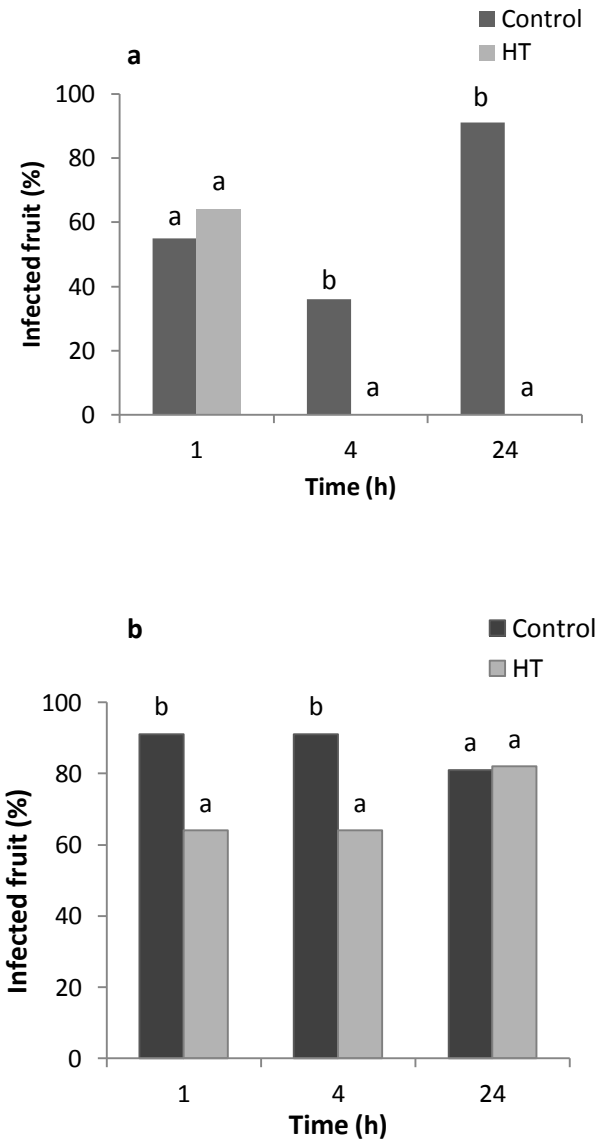


Figure 2 Effect of hot water treatment (45°C) against *Penicillium expansum* rots on artificially infected fruit on laboratory trials (post-HT) (a). The duration of the treatment was 10 min applied on artificially infected fruit with 20 µL of 10^4 conidia suspension of *P. expansum* after 1, 4 and 24 hours. The effect of hot water treatment (45°C) against *Penicillium expansum* rots on artificially infected fruit on laboratory trials was detected in pre-HT (b). The duration of the treatment was 10 min applied on fruit. Treated apples were consequently wounded and inoculated with 20 µL of 10^4 conidia suspension of *P. expansum* after 1, 4 and 24 hours from the treatment. Each biological replicate consisted of five fruits for three replicates, the experiment was repeated twice. Data on pathogenicity and fruit quality parameters were analyzed for significant differences *($P<0.05$)* by analysis of variance (ANOVA) using LSD test.

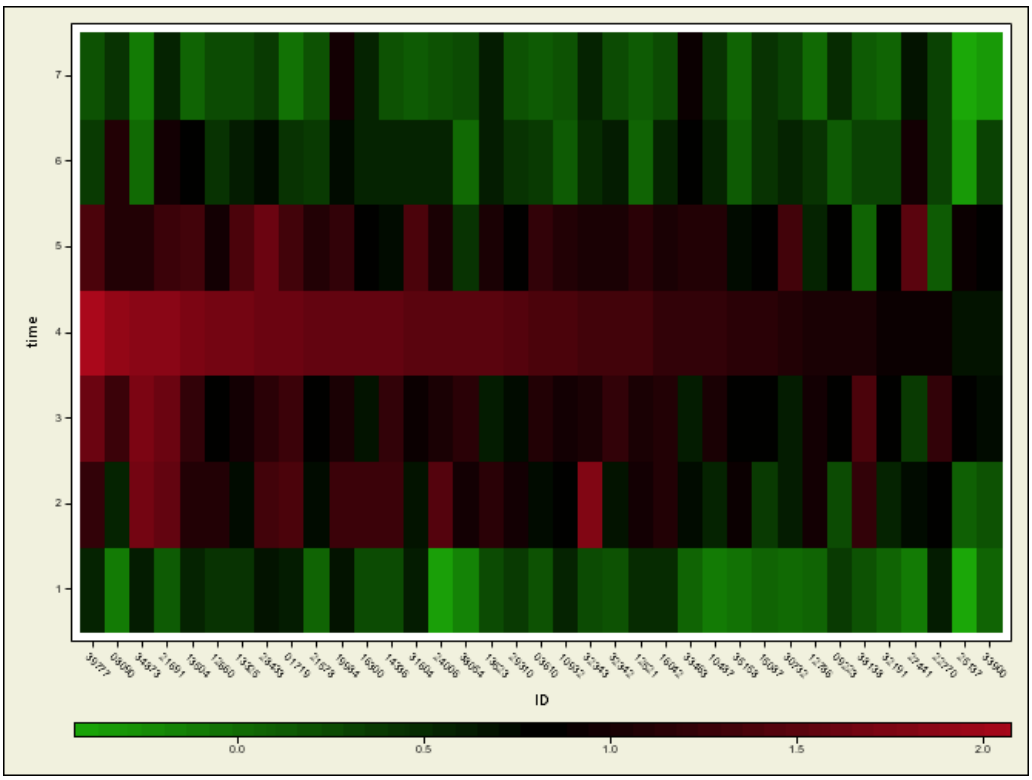
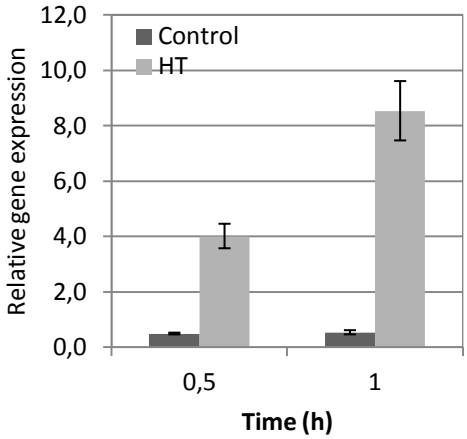
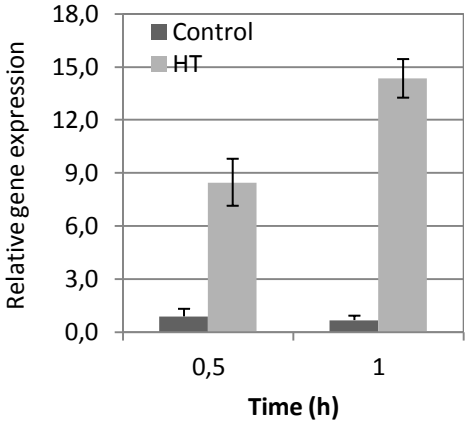


Figure 3 Hierarchical clustering of differentially expressed genes in the two treatments (control and HT) during seven time points (0, 15, 30 minutes, 1, 4, 8, 24 hours) that determined to be significantly different (*P <0.05*) using Qlucore Omics Explorer (Qlucore, Lund, Sweden) software.

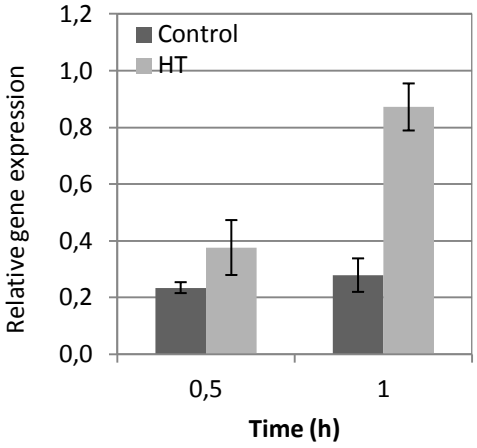
hsp STI



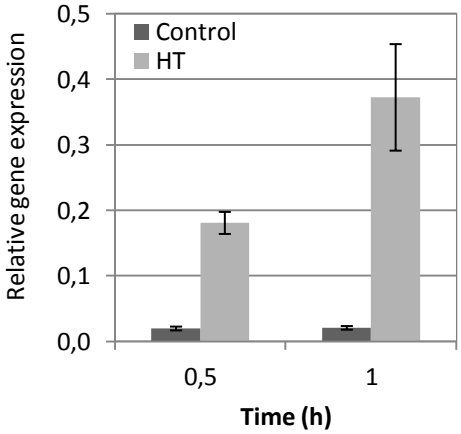
hsp 18.5 kDa



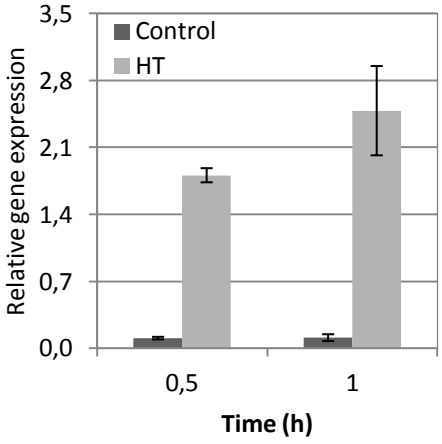
Granule-bond starch synthase-2



1-aminocyclopropane-1-carboxylate oxidase homolog 1



Glycogenin-1



Aspartate-semialdehyde dehydrogenase

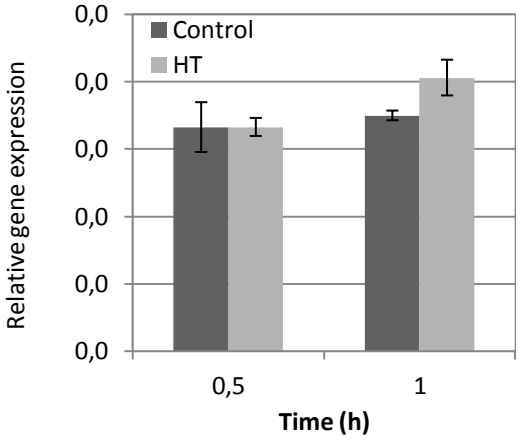


Figure 4 Relative expression levels of *HSP-STI* and *HSP 18.5 kDa, Granule-bond starch synthase-2, 1-aminocyclopropane-1-carboxylate oxidase homolog 1,Glycogenin-1* and *Aspartate-semialdehyde dehydrogenase* genes in heat treated and untreated apples tissues. Treated fruit were dipped in water at 45°C for 10 min and control fruit were dipped in water at room temperature for 10 min. Data refer to 0.5 (30 min) and 1 h after treatment. All values were normalized using *actin* housekeeping gene. Each value is the mean of 3 replicates of 3 fruits each $\pm$ st dv.

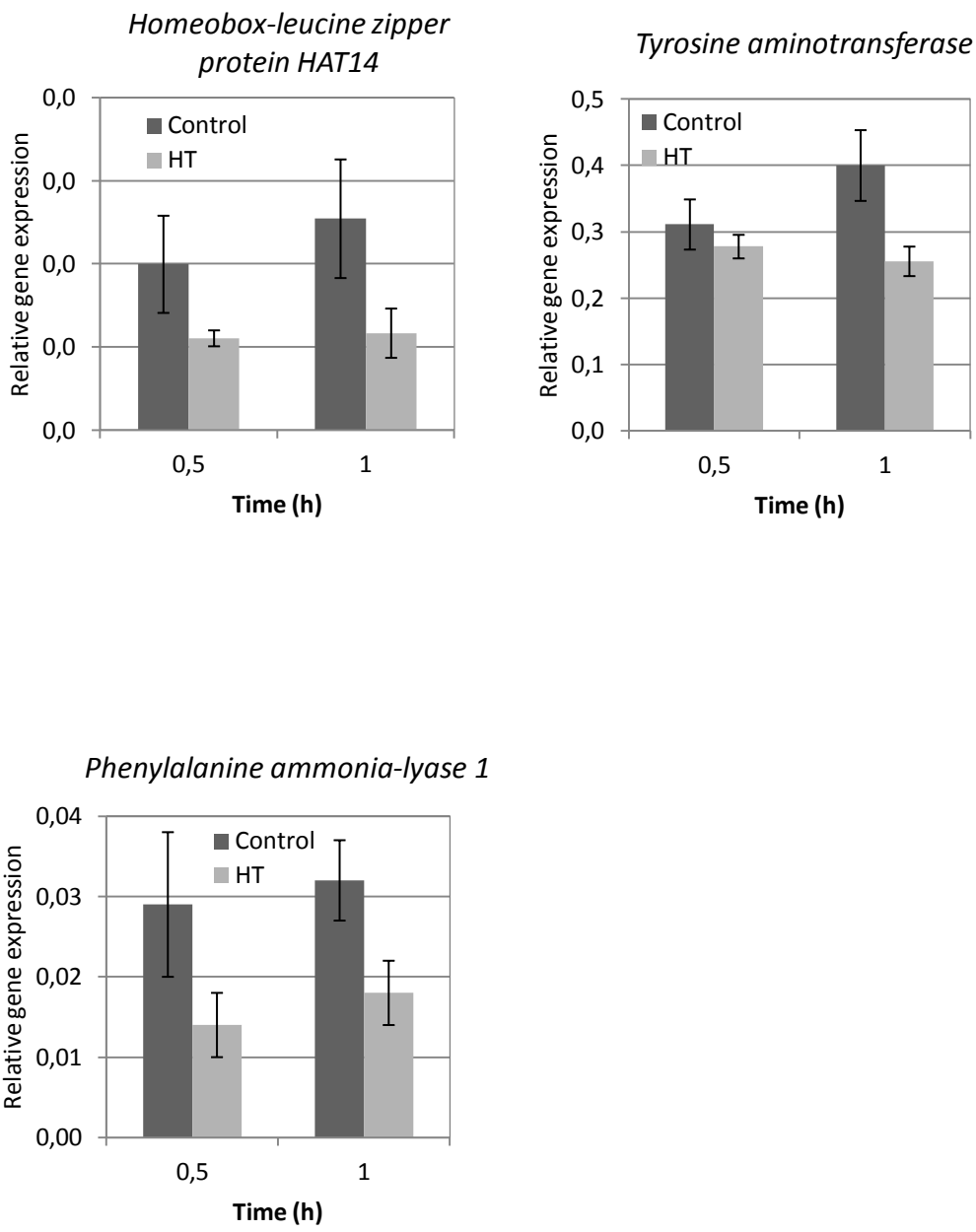


Figure 5

Relative expression levels of *Homeobox-leucine zipper protein HAT14* and *Tyrosine aminotransferase, Phenylalanine ammonia-lyase 1* genes in heat treated and untreated apples tissues. Treated fruit were dipped in water at 45°C for 10 min and control fruit were dipped in water at room temperature for 10 min. Data refer to 0.5 (30 min) and 1 h after treatment. All values were normalized using *actin* housekeeping gene. Each value is the mean of 3 replicates of 3 fruits each ± st dv.

5.7 Tables

Table 1 Primers used in qRT-PCR reactions

Gene Name	Accession N°	Primer sequences (5'-3')	Annealing temperature (°C)
Aspartate-semialdehyde dehydrogenase	MDP0000220526	F: TAATGGCTGTCACCCCTCTC R: TTGCAGCTCAAGCTCTCTCA	58
1-aminocyclopropane-1-carboxylate oxidase homolog 1	MDP0000878773	F: ATGCGGAGAACAATGAAAGG R: CATGACGTCAAGTGGAATGG	56
Glycogenin-1	MDP0000466683	F: GAATCTCAGGGTTGCATCGT R: AATGCGCAGCTTCGAGTAGT	57
Granule-bound starch synthase 2, chloroplastic/amyloplastic	MDP0000842179	F: CATTGTTGCCGGTGTATTTG R:CGACCCTGGTGAGCTATGTT	57
Heat shock protein 18.5	MDP0000207407	F: ATTCACACCCAGCAAAAACC R: GGTCCCAGATGTCCAGAGAG	57
Heat shock protein STI	MDP0000161691	F: CATCGCAACCCAGATACCTT R: CCTTTCTCACGCTCCTCATC	58
Homeobox-leucine zipper protein HAT14	MDP0000135540	F: TCCTCGTAGCCACGTTTCTT R: AAACCTATTCCCGGTCCATC	57
Phenilalanine ammonia lyase-1	MDP0000388769	F: CTTGGAAGCCATCACCAAG R: AAACCGGCAATGTAGGACAG	57
Tyrosine aminotransferase	MDP0000180890	F: GGAACAGCAGTAGGGCTGAC R: AATTTTCTGGCATGCCTTTG	57
Actin		F:CTATGTTCCCTGGTATTGCGACC R: GCCACAACCTTGTTTTTCATGC	57

Table 2

Transcripts differentially expressed in HT fruit compare to the control ones immediately after the treatment (time 0).

MDP	Description	Ratio	GO code	GO terms	p value	*Arabidopsis* protein
Upregulated genes						
MDP0000155376	heavy-metal-associated domain-containing protein	3,3	GO:0005634 GO:0046872	nucleus metal ion binding	7.9 e-40	AT1G01490
MDP0000314161	Cyclin-A2-3	2,3	GO:0008283 GO:0000911 GO:0042023 GO:0010311 GO:0000226 GO:0000278 GO:0045736 GO:0000280 GO:2000123 GO:0043248 GO:0043161 GO:0051726 GO:0006275 GO:0010389 GO:0051510 GO:0051788 GO:0051225 GO:0010440 GO:0005634 GO:0016538 GO:0005515	cell proliferation cytokinesis by cell plate formation DNA endoreduplication lateral root formation microtubule cytoskeleton organization mitotic cell cycle negative regulation of cyclin-dependent protein serine/threonine kinase activity nuclear division positive regulation of stomatal complex development proteasome assembly proteasome-mediated ubiquitin-dependent protein catabolic process regulation of cell cycle regulation of cell cycle regulation of G2/M transition of mitotic cell cycle regulation of unidimensional cell growth response to misfolded protein spindle assembly stomatal lineage progression nucleus cyclin-dependent protein serine/threonine kinase regulator activity protein binding	4.9e-26	CYCLIN A2;3
MDP0000318167	Zinc finger protein CONSTANS-LIKE 4	2,0	GO:0008150 GO:0009827 GO:0009860 GO:0005634 GO:0003674	biological process plant-type cell wall modification pollen tube growth nucleus molecula function	1.4 e-78	AT1G04500
MDP0000323296	18.5 kDa class I heat shock protein	2,0	GO:0010286 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005737	heat acclimation protein folding response to heat response to high light intensity response to hydrogen peroxide cytoplasm	3.0 e-51	hsp18.2
MDP0000292810	Putative disease resistance protein RGA4	1,8	GO:0006952 GO:0005634	defense response nucleus	5 e-65	AT3G14460
MDP0000249811	Pre-mRNA-processing factor 40 homolog B	1,8	GO:0008380 GO:0005829 GO:0016592 GO:0005634 GO:0005886 GO:0070063	RNA splicing cytosol mediator complex nucleus plasma membrane RNA polymerase binding	3.2e-184	PRP40A, pre-mRNA-processing protein 40
MDP0000253143	Probable nucleoredoxin 2	1,8	GO:0009860 GO:0010183 GO:0080092	pollen tube growth pollen tube guidance regulation of pollen tube growth	4.2e-74	AT1G60420

			GO:0046686 GO:0005829	response to cadmium ion cytosol		
MDP0000577510	ABC transporter E family member 2	1,8	GO:0051604 GO:0005215	protein maturation transporter activity	4.5e-128	AT4G19210
MDP0000176307	Proteasome subunit beta type-5	1,8	GO:0006511 GO:0005634 GO:0000502 GO:0005839 GO:0004175	ubiquitin-dependent protein catabolic process nucleus proteasome complex proteasome core complex endopeptidase activity	1.7e-117	AT3G26340
MDP0000483080	Early nodulin-93	1,8	GO:0009723 GO:0005739 GO:0003674	response to ethylene mitochondrion molecular function	6.3e-12	AT5G25940
MDP0000258605	REF4 (REDUCED EPIDERMAL FLUORESCENCE 4)	1,7	GO:0045010 GO:0007155 GO:0071555 GO:2000762 GO:0048765 GO:0010090 GO:0016592 GO:0003674	actin nucleation cell adhesion cell wall organization regulation of phenylpropanoid metabolic process root hair cell differentiation trichome morphogenesis mediator complex molecular function		RFR1
MDP0000444367	Kinesin-related protein 4	1,7	GO:0005737 GO:0009506 GO:0003777	cytoplasm plasmodesma microtubule motor activity	2.9e-213	AT5G66310
MDP0000166956	Methionine aminopeptidase 1A	1,7	GO:0043481 GO:0048440 GO:0031365 GO:0016485 GO:0010048 GO:0005737 GO:0005829	anthocyanin accumulation in tissues in response to UV light carpel development N-terminal protein amino acid modification protein processing vernalization response cytoplasm cytosol	7.4e-143	methionine aminopeptidase 1A
MDP0000237848	Protein DCL, chloroplastic	1,7	GO:0008150 GO:0009507 GO:0003674	biological process chloroplast molecular function	3.2e-59	AT1G45230
MDP0000593607	LRR receptor-like serine/threonine-protein kinase EFR	1,7	GO:0010204 GO:0042742 GO:0050832 GO:0016045 GO:0009595 GO:0009581 GO:0002764 GO:0009867 GO:0000165 GO:0031348 GO:0009626 GO:0006468 GO:0006612 GO:0010359 GO:0031347 GO:0010310 GO:0050776 GO:0043900 GO:0010363 GO:0010200 GO:0009697 GO:0009862	defense response signaling pathway, resistance gene-independent defense response to bacterium defense response to fungus detection of bacterium detection of biotic stimulus detection of external stimulus immune response-regulating signaling pathway jasmonic acid mediated signaling pathway MAPK cascade negative regulation of defense response plant-type hypersensitive response protein phosphorylation protein targeting to membrane regulation of anion channel activity regulation of defense response regulation of hydrogen peroxide metabolic process regulation of immune response regulation of multi-organism process regulation of plant-type hypersensitive response response to chitin vernalization response systemic acquired resistance, salicylic acid mediated signaling pathway	9.8 e-25	AT5G20480, EFR

			GO:0007169 GO:0005886 GO:0005524 GO:0016301 GO:0005515 GO:0004674 GO:0019199	transmembrane receptor protein tyrosine kinase signaling pathway plasma membrane ATP binding kinase activity protein binding protein serine/threonine kinase activity transmembrane receptor protein kinase activity		
Downregulated genes						
MDP0000322577	Unknown protein	0,5	GO:0010090 GO:0005886 GO:0003674	trichome morphogenesis plasma membrane molecular function	1,20E-35	AT1G64690 BLT
MDP0000142904	Protein kinase G11A	0,5	GO:0009855 GO:0010014 GO:0010073 GO:0006346 GO:0016246 GO:0005634 GO:0005886 GO:0016301 GO:0005515	determination of bilateral symmetry meristem initiation meristem maintenance methylation-dependent chromatin silencing RNA interference nucleus plasma membrane kinase activity protein binding	1.8e-213	AT2G36350
MDP0000205276	ATPase family AAA domain-containing protein 1-A	0,4	GO:0005886 GO:0016887	plasma membrane ATPase activity	5.5e-158	AT4G27680

Table 3

Transcripts differentially expressed in HT fruit compare to the control ones after 15 minutes from the treatment.

MDP	Description	Ratio	Go code	GO terms	p value	*Arabidopsis* protein
Upregulated genes						
MDP0000543224	Probable pyridoxal biosynthesis protein PDX1.2	3,3	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0042819 GO:0005737 GO:0005829 GO:0005515 GO:0046982	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide vitamin B6 biosynthetic process cytoplasm cytosol protein binding protein heterodimerization activity	1.1e-111	PDX1.2, pyridoxine biosynthesis 1.2
MDP0000327491	Cleavage and polyadenylation specificity factor subunit 5	3,3	GO:0008150 GO:0005634 GO:0005515	biological process nucleus protein binding	1.4e-98	AT4G25550
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	3,2	GO:0016049 GO:0009793 GO:0007030 GO:0048507 GO:0009933 GO:0045053 GO:0010016 GO:0005829 GO:0005794 GO:0003674	cell growth embryo development ending in seed dormancy Golgi organization meristem development meristem structural organization protein retention in Golgi apparatus shoot system morphogenesis cytosol Golgi apparatus molecular function	2.5e-167	EYE
MDP0000290546	DnaJ homolog subfamily B member 13	3,1	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm	4.1e-120	AT2G20560
MDP0000684170	Heat shock cognate 70 kDa protein 4	2,9	GO:0010286 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005737	heat acclimation protein folding response to heat response to high light intensity response to hydrogen peroxide cytoplasm	7.2e-217	heat-shock protein 70T-2
MDP0000707429	transferase, transferring glycosyl groups	2,8	GO:0005576	extracellular region	6.0e-105	AT3G18180
MDP0000145643	REF/SRPP-like protein	2,6	GO:0008150 GO:0005737 GO:0005773	biological process cytoplasm vacuole	8.4e-54	AT1G67360
MDP0000302297	Somatic embryogenesis receptor kinase 1	2,5	GO:0006355 GO:0005634 GO:0003700 GO:0008270	regulation of transcription, DNA-templated nucleus sequence-specific DNA binding transcription factor activity zinc ion binding	1.4e-75	B-box domain protein 12, BBX12
MDP0000207407	18.5 kDa class I heat shock protein	2,4	GO:0010286 GO:0006457 GO:0034976 GO:0009408 GO:0009644	heat acclimation protein folding response to endoplasmic reticulum stress response to heat response to high light intensity	5.3e-42	AT2G29500

			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000254930	PPPDE peptidase domain-containing protein 2	2,4	GO:0008150	biological process	8.4e-90	AT3G07090
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000303015	Heat shock protein 101	2,4	GO:0010286	heat acclimation	0	AT1G74310 HSP101
			GO:0045727	positive regulation of translation		
			GO:0006457	protein folding		
			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
MDP0000172863	Probable inactive receptor kinase	2,3	GO:0006468	protein phosphorylation	1.6e-208	AT1G27190
			GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway		
			GO:0005576	extracellular region		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0005524	ATP binding		
			GO:0016301	kinase activity		
			GO:0004674	protein serine/threonine kinase activity		
MDP0000574524	17.5 kDa class I heat shock protein	2,3	GO:0010286	heat acclimation	6.1e-28	AT2G29500
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000226817	EGY3 (ethylene-dependent gravitropism-deficient and yellow-green-like 3)	2,3	GO:0006457	protein folding	3.8e-206	EGY3 ETHYLENE-DEPENDENT GRAVITROPISM-DEFICIENT AND YELLOW-GREEN-LIKE 3
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
MDP0000657396	Protein FAM32A	2,3	GO:0008150	biological process	1.8e-18	AT1G16810
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000314161	Cyclin-A2-3	2,3	GO:0008283	cell proliferation	4.9e-26	CYCLIN A2;3
			GO:0000911	cytokinesis by cell plate formation		
			GO:0042023	DNA endoreduplication		
			GO:0010311	lateral root formation		
			GO:0000226	microtubule cytoskeleton organization		
			GO:0000278	mitotic cell cycle		
			GO:0045736	negative regulation of cyclin-dependent protein serine/threonine kinase activity		
			GO:0000280	nuclear division		
			GO:2000123	positive regulation of stomatal complex development		
			GO:0043248	proteasome assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0051726	regulation of cell cycle		
			GO:0006275	regulation of cell cycle		
			GO:0010389	regulation of G2/M transition of mitotic cell cycle		

			GO:0051510	regulation of unidimensional cell growth		
			GO:0051788	response to misfolded protein		
			GO:0051225	spindle assembly		
			GO:0010440	stomatal lineage progression		
			GO:0005634	nucleus		
			GO:0016538	cyclin-dependent protein serine/threonine kinase regulator activity		
			GO:0005515	protein binding		
MDP0000220754	unknown protein	2,3	GO:0008150	biological process	1.4e-128	AT4G36440
			GO:0003674	molecular function		
MDP0000153707	Nuclease PA3	2,3	GO:0006308	DNA catabolic process	5.2e-115	BFN1
			GO:0080187	floral organ senescence		AT1G11190
			GO:0010150	leaf senescence		
			GO:0005576	extracellular region		
			GO:0016891	endoribonuclease activity, producing 5'-phosphomonoesters		
			GO:0003676	nucleic acid binding		
			GO:0000014	single-stranded DNA endodeoxyribonuclease activity		
			GO:0043765	T/G mismatch-specific endonuclease activity		
MDP0000311339	Heat shock 70 kDa protein	2,3	GO:0010286	heat acclimation	2.4e-59	Hsp70b
			GO:0006457	protein folding		heat shock protein 70B
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009615	response to virus		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0016020	membrane		
			GO:0005886	plasma membrane		
MDP0000466683	Glycogenin-1	2,2	GO:0016051	carbohydrate biosynthetic process	1.5e-148	AT2G47180
			GO:0006012	galactose metabolic process		
			GO:0009737	response to abscisic acid		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxid		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009414	response to water deprivation		
			GO:0005575	cellular component		
			GO:0047216	inositol 3-alpha-galactosyltransferase activity		
			GO:0016758	transferase activity, transferring hexosyl groups		
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	2,1	GO:0016049	cell growth	2.5e-167	AT5G51430
			GO:0009793	embryo development ending in seed dormancy		
			GO:0007030	Golgi organization		
			GO:0048507	meristem development		
			GO:0009933	meristem structural organization		
			GO:0045053	protein retention in Golgi apparatus		
			GO:0010016	shoot system morphogenesis		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0003674	molecular function		

MDP0000291335	Tetratricopeptide repeat protein 4 homolog	2,1	GO:0005737 GO:0051879	cytoplasm Hsp90 protein binding	2.6e-74	TPR2 tetratricopeptide repeat 2
MDP0000122734	Heat shock cognate 70 kDa protein 4	2,1	GO:0006457 GO:0016567 GO:0009617 GO:0046686 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0009266 GO:0009615 GO:0048046 GO:0005618 GO:0005829 GO:0005794 GO:0005739 GO:0005886 GO:0005774 GO:0005524 GO:0031625	protein folding protein ubiquitination response to bacterium response to cadmium ion response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide response to temperature stimulus response to virus apoplast cell wall cytosol Golgi apparatus mitochondrion plasma membrane vacuolar membrane ATP binding ubiquitin protein ligase binding	1.8e-256	AT3G12580
MDP0000351085	Protein RUPTURED POLLEN GRAIN 1	2,1	GO:0008150 GO:0005887 GO:0016020 GO:0005886 GO:0051119	biological process integral component of plasma membrane membrane plasma membrane sugar transmembrane transporter activity	4.7e-73	SWEET9
MDP0000171695	unknown protein	2,1	GO:0009061 GO:0006979 GO:0005634 GO:0003674	anaerobic respiration response to oxidative stress nucleus molecular function	1.9e-30	AT3G10020
MDP0000465593	E3 ubiquitin-protein ligase RING1-like	2,0	GO:0005634 GO:0008270	nucleus zinc ion binding	5.6e-27	AT1G26800
MDP0000243895	Heat shock factor protein HSF30	2,0	GO:0034605 GO:0071456 GO:0034620 GO:0010286 GO:0045893 GO:0006457 GO:0010200 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0001666 GO:0009407 GO:0009507 GO:0005634 GO:0003677 GO:0005515 GO:0003700	cellular response to heat cellular response to hypoxi cellular response to unfolded protein heat acclimation positive regulation of transcription, DNA-templated protein folding response to chitin response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide response to hypoxia toxin catabolic process chloroplast nucleus DNA binding protein binding sequence-specific DNA binding transcription factor activity	3.9e-91	AT2G26150
MDP0000185280	Cellulose synthase-like protein G3	2,0	GO:0000271 GO:0005886 GO:0016759 GO:0016740	polysaccharide biosynthetic process plasma membrane cellulose synthase activity transferase activity	1.7e-233	AT4G23990

			GO:0016757	transferase activity, transferring glycosyl groups		
MDP0000275263	THO complex subunit 4	2,0	GO:0008150 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0003674	biological process protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm cytosol molecular function	2.6e-61	AT1G66080
MDP0000161691	Heat shock protein STI	2,0	GO:0009165 GO:0046686 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0005634 GO:0005886	nucleotide biosynthetic process response to cadmium ion response to high light intensity response to hydrogen peroxide cytoplasm cytosol nucleus plasma membrane	2.1e-213	Hop2
MDP0000700383	17.9 kDa class II heat shock protein	2,0	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm	8.8e-35	HSP17.6II 17.6 kDa class II heat shock protein
MDP0000432871	Prohibitin-2	2,0	GO:0005747 GO:0005739 GO:0005774	mitochondrial respiratory chain complex I mitochondrion vacuolar membrane	1.0e-109	AT4G28510
MDP0000153978	ATBAG5 (arabidopsis thaliana bcl-2-associated athanogene 5); protein binding	2,0	GO:0050832 GO:0010286 GO:0012502 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0010228 GO:0005634 GO:0009506 GO:0005516	defense response to fungus heat acclimation induction of programmed cell death protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide vegetative to reproductive phase transition of meristem nucleus plasmodesma calmodulin binding	2.9e-12	BAG6 BCL-2-associated athanogene 6
MDP0000744777	Protein popC	1,9	GO:0006952	defense response	2.2e-62	AT3G25510
MDP0000795157	DnaJ homolog subfamily B member 4	1,9	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm	1.3e-114	AT2G20560
MDP0000256438	Small heat shock protein, chloroplastic	1,9	GO:0010286 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0009507	heat acclimation protein folding response to heat response to high light intensity response to hydrogen peroxide chloroplast	1.4e-60	AT4G25200
MDP0000323296	18.5 kDa class I heat shock protein	1,9	GO:0010286 GO:0006457 GO:0009408	heat acclimation protein folding response to heat	3.0 e-51	hsp18.2

			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000196824	Probably inactive leucine-rich repeat receptor-like protein kinase	1,9	GO:0006952	defense response	7.2e-70	RLP35 AT3G11080
			GO:0007165	signal transduction		
			GO:0016301	kinase activity		
MDP0000125300	Small heat shock protein, chloroplastic	1,9	GO:0006457	protein folding	1.4e-36	HSP23.6-MITO AT4G25200
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000271360	Universal stress protein A-like protein	1,9	GO:0002238	response to molecule of fungal origin	2.5e-53	AT2G47710
			GO:0005794	Golgi apparatus		
			GO:0005886	plasma membrane		
			GO:0005773	vacuole		
			GO:0003674	molecular function		
MDP0000202279	Probable protein phosphatase 2C 26	1,8	GO:0071482	cellular response to light stimulus	6.9e-79	PBCP PHOTOSYSTEM II CORE PHOSPHATASE
			GO:0035970	peptidyl-threonine dephosphorylation		
			GO:0010207	photosystem II assembly		
			GO:0009657	plastid organization		
			GO:0046777	protein autophosphorylation		
			GO:0010155	regulation of proton transport		
			GO:0006364	rRNA processing		
			GO:0010027	thylakoid membrane organization		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
MDP0000122734	Heat shock cognate 70 kDa protein 4	1,8	GO:0006457	protein folding	1.8e-256	AT3G12580
			GO:0016567	protein ubiquitination		
			GO:0009617	response to bacterium		
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009266	response to temperature stimulus		
			GO:0009615	response to virus		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005739	mitochondrion		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
			GO:0031625	ubiquitin protein ligase binding		
MDP0000768256	Hsp70-binding protein 1	1,8	GO:0006457	protein folding	2.7e-132	Fes1A
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009651	response to salt stress		
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0030544	Hsp70 protein binding		
MDP0000446914	Glycogenin-1	1,8	GO:0016051	carbohydrate biosynthetic process	1.1e-148	AT2G47180
			GO:0006012	galactose metabolic process		
			GO:0009737	response to abscisic acid		

			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009414	response to water deprivation		
			GO:0005575	cellular component		
			GO:0047216	inositol 3-alpha-galactosyltransferase activity		
			GO:0016758	transferase activity, transferring hexosyl groups		
MDP0000119940	Probable protein phosphatase 2C 13	1,8	GO:0004722	protein serine/threonine phosphatase activity	3.4e-100	AT1G48040
MDP0000119199	Heat stress transcription factor A-6b	1,7	GO:0009407	toxin catabolic process	2.1e-82	AT3G22830
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000873379	Arginine biosynthesis bifunctional protein ArgJ 1	1,7	GO:0006526	arginine biosynthetic process	6.6e-127	AT2G37500
			GO:0009220	pyrimidine ribonucleotide biosynthetic process		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005739	mitochondrion		
			GO:0004358	glutamate N-acetyltransferase activity		
MDP0000120125	Probable galacturonosyltransferase-like 10	1,7	GO:0009738	abscisic acid-activated signaling pathway	2.4e-123	GATL10
			GO:0016051	carbohydrate biosynthetic process		
			GO:0030968	endoplasmic reticulum unfolded protein response		
			GO:0035556	intracellular signal transduction		
			GO:0006979	response to oxidative stress		
			GO:0005794	Golgi apparatus		
			GO:0047262	polygalacturonate 4-alpha-galacturonosyltransferase activity		
			GO:0016758	transferase activity, transferring hexosyl groups		
MDP0000310899	unknown protein	1,7	GO:0008150	biological process	5.2e-42	AT1G72510
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000202749	THO complex subunit 4	1,7	GO:0008150	biological process	4.2e-77	AT1G66080
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0003674	molecular function		
MDP0000878181	unknown protein	1,7	GO:0008150	biological process	3.3e-09	AT1G32928
			GO:0005575	cellular component		
			GO:0003674	molecular function		
MDP0000925901	Heat stress transcription factor A-2b	1,7	GO:0009407	toxin catabolic process	1.1e-49	AT3G22830
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000873667	Brassinosteroid-regulated protein BRU1	1,7	GO:0005576	extracellular region	2.9e-105	XTH16
			GO:0016798	hydrolase activity, acting on glycosyl bonds		

MDP0000720039	Polyubiquitin	1,7	GO:0006464 GO:0042787 GO:0006511	cellular protein modification process protein ubiquitination involved in ubiquitin-dependent protein catabolic process ubiquitin-dependent protein catabolic process	1.4e-135	AT5G20620
MDP0000205027	Putative uncharacterized protein	1,7	GO:0008150 GO:0003674	biological process molecular function	9.3e-12	AT2G24395
MDP0000241447	21 kDa protein	1,7	GO:0006096 GO:0007030 GO:0006972 GO:0046686 GO:0009651 GO:0009266 GO:0006833 GO:0046910	glycolysis Golgi organization hyperosmotic response response to cadmium ion response to salt stress response to temperature stimulus water transport pectinesterase inhibitor activity	1.3e-50	AT5G62350
MDP0000322220	Heat shock cognate 70 kDa protein 1	1,7	GO:0042742 GO:0050832 GO:0006094 GO:0006096 GO:0010187 GO:0006457 GO:0046686 GO:0009409 GO:0009408 GO:0009651 GO:0009615 GO:0090332 GO:0048046 GO:0005618 GO:0009507 GO:0005737 GO:0005829 GO:0022626 GO:0005794 GO:0016020 GO:0005730 GO:0005634 GO:0005886 GO:0009506 GO:0005774 GO:0002020 GO:0005515	defense response to bacterium defense response to fungus gluconeogenesis glycolysis negative regulation of seed germination protein folding response to cadmium ion response to cold response to heat response to salt stress response to virus stomatal closure apoplast cell wall chloroplast cytoplasm cytosol cytosolic ribosome Golgi apparatus membrane nucleolus nucleus plasma membrane plasmodesma vacuolar membrane protease binding protein binding	1.7e-298	HSC70-1
MDP0000667723	Acylphosphatase	1,7	GO:0009507 GO:0003998	chloroplast acylphosphatase activity	3.7e-32	AT5G03370
MDP0000549793	oxidoreductase	1,7	GO:0009688 GO:0034605 GO:0010286 GO:0009860 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005788 GO:0009506 GO:0016491	abscisic acid biosynthetic process cellular response to heat heat acclimation pollen tube growth protein folding response to heat response to high light intensity response to hydrogen peroxide endoplasmic reticulum lumen plasmodesma oxidoreductase activity	4.9e-131	ATERDJ3A AT3G08970
MDP0000205027	Putative uncharacterized protein	1,7	GO:0008150 GO:0003674	biological process molecular function	9.3e-12	AT2G24395
MDP0000271554	HSA32	1,7	GO:0010286 GO:0008152	heat acclimation metabolic process	7.3e-114	AT4G21320

			GO:0010608	posttranscriptional regulation of gene expression		
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005618	cell wall		
			GO:0005634	nucleus		
			GO:0004252	serine-type endopeptidase activity		
Downregulated genes						
MDP0000323212	Auxin-repressed 12.5 kDa protein	0,5	GO:0005634	nucleus	2.0e-24	DRM2
MDP0000551283	Probable WRKY transcription factor 19	0,5	GO:0008150	biological process	1.9e-190	AT1G64140
			GO:0005576	extracellular region		
			GO:0003674	molecular function		
MDP0000135486	D111/G-patch domain-containing protein	0,5	GO:0008150	biological process	6.9e-41	AT1G63980
			GO:0005622	intracellular		
			GO:0005634	nucleus		
			GO:0003676	nucleic acid binding		
MDP0000688187	Universal stress protein A-like protein	0,5	GO:0010106	cellular response to iron ion starvation	3.7e-23	AT1G09740
			GO:0006826	iron ion transport		
			GO:0015706	nitrate transport		
			GO:0002238	response to molecule of fungal origin		
			GO:0010167	response to nitrate		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000149535	Myb-related protein 306	0,5	GO:0006355	regulation of transcription, DNA-templated	2.0e-79	AT3G47600
			GO:0009737	response to abscisic acid		
			GO:0009733	response to auxin		
			GO:0046686	response to cadmium ion		
			GO:0009723	response to ethylene		
			GO:0009753	response to jasmonic acid		
			GO:0080167	response to karrikin		
			GO:0009751	response to salicylic acid		
			GO:0009651	response to salt stress		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000272025	Probable hexaprenyl pyrophosphate synthase, mitochondrial	0,5	GO:0006635	fatty acid beta-oxidation		
			GO:0016558	protein import into peroxisome matrix		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0009524	phragmoplast		
MDP0000517257	Ethylene-responsive transcription factor 2	0,5	GO:0009738	abscisic acid-activated signaling pathway		
			GO:0009873	ethylene-activated signaling pathway		
			GO:0042538	hyperosmotic salinity response		
			GO:0035556	intracellular signal transduction		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006355	regulation of transcription, DNA-templated		
			GO:0002679	respiratory burst involved in defense response		
			GO:0009737	response to abscisic acid		
			GO:0010200	response to chitin		
			GO:0009723	response to ethylene		

			GO:0009414 GO:0005643 GO:0005634 GO:0003677 GO:0003700	response to water deprivation nuclear pore nucleus DNA binding sequence-specific DNA binding transcription factor activity		

Table 4

Transcripts differentially expressed in HT fruit compare to the control after 30 minutes from the treatment.

MDP	Description	Ratio	Go code	GO terms	*p value*	*Arabidopsis* protein
Upregulated genes						
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	3,3	GO:0016049 GO:0009793 GO:0007030 GO:0048507 GO:0009933 GO:0045053 GO:0010016 GO:0005829 GO:0005794 GO:0003674	cell growth embryo development ending in seed dormancy Golgi organization meristem development meristem structural organization protein retention in Golgi apparatus shoot system morphogenesis cytosol Golgi apparatus molecular function	2.5e-167	AT5G51430
MDP0000684170	Heat shock cognate 70 kDa protein 4	3,1	GO:0010286 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005737	heat acclimation protein folding response to heat response to high light intensity response to hydrogen peroxide cytoplasm	7.2e-217	heat-shock protein 70T-2
MDP0000657396	Protein FAM32A	3,0	GO:0008150 GO:0005634 GO:0003674	biological process nucleus molecular function	1.8e-18	AT1G16810
MDP0000220754	unknown protein	2,6	GO:0008150 GO:0003674	biological process molecular function	1.4e-128	AT4G36440
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	2,6	GO:0016049 GO:0009793 GO:0007030 GO:0048507 GO:0009933 GO:0045053 GO:0010016 GO:0005829 GO:0005794 GO:0003674	cell growth embryo development ending in seed dormancy Golgi organization meristem development meristem structural organization protein retention in Golgi apparatus shoot system morphogenesis cytosol Golgi apparatus molecular function	2.5e-167	AT5G51430
MDP0000290546	DnaJ homolog subfamily B member 13	2,5	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm	4.1e-120	AT2G20560
MDP0000543224	Probable pyridoxal biosynthesis protein PDX1.2	2,5	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0042819 GO:0005737 GO:0005829 GO:0005515 GO:0046982	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide vitamin B6 biosynthetic process cytoplasm cytosol protein binding protein heterodimerization activity	1.1e-111	PDX1.2, pyridoxine biosynthesis 1.2
MDP0000456824	Chaperone protein dnaJ11, chloroplastic	2,5	GO:0006457	protein folding	2.1e-23	AT3G13310

MDP0000370184	Putative uncharacterized protein	2,4	GO:0008150 GO:0003674	biological process molecular function	0.00032	AT4G25170
MDP0000209621	unknown protein	2,4	GO:0008150 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005739 GO:0005634 GO:0003674	biological process protein folding response to heat response to high light intensity response to hydrogen peroxide mitochondrion nucleus molecular function	7.7e-41	AT5G47830
MDP0000145643	REF/SRPP-like protein	2,4	GO:0008150 GO:0005737 GO:0005773	biological process cytoplasm vacuole	8.4e-54	AT1G67360
MDP0000161691	Heat shock protein STI	2,4	GO:0009165 GO:0046686 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0005634 GO:0005886	nucleotide biosynthetic process response to cadmium ion response to high light intensity response to hydrogen peroxide cytoplasm cytosol nucleus plasma membrane	2.1e-213	Hop2
MDP0000235765	Chaperonin CPN60-2, mitochondrial	2,4	GO:0051131 GO:0007005 GO:0006457 GO:0006626 GO:0046686 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005829 GO:0022626 GO:0005759 GO:0005739 GO:0005774 GO:0005524 GO:0005507	chaperone-mediated protein complex assembly mitochondrion organization protein folding protein targeting to mitochondrion response to cadmium ion response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytosol cytosolic ribosome mitochondrial matrix mitochondrion vacuolar membrane ATP binding copper ion binding	2.1e-253	HSP60
MDP0000153978	protein binding	2,4	GO:0050832 GO:0010286 GO:0012502 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0010228 GO:0005634 GO:0009506 GO:0005516	defense response to fungus heat acclimation induction of programmed cell death protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide vegetative to reproductive phase transition of meristem nucleus plasmodesma calmodulin binding	2.9e-12	BAG6 BCL-2-associated athanogene 6
MDP0000878181	unknown protein	2,3	GO:0008150 GO:0005575 GO:0003674	biological process cellular component molecular function	3.3e-09	AT1G32928
MDP0000872073	Putative uncharacterized protein	2,3	GO:0008150 GO:0005575 GO:0003674	biological process cellular component molecular function	0.0093	orf19.4959
MDP0000254930	PPPDE peptidase domain-containing	2,3	GO:0008150 GO:0005737	biological process cytoplasm	8.4e-90	AT3G07090

	protein 2		GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000158045	EXO (EXORDIUM)	2,3	GO:0009741	response to brassinosteroid	7.5e-110	AT4G08950
			GO:0016126	sterol biosynthetic process		
			GO:0005618	cell wall		
			GO:0005576	extracellular region		
			GO:0005794	Golgi apparatus		
			GO:0009505	plant-type cell wall		
			GO:0003674	molecular function		
MDP0000225534	Purple acid phosphatase 23	2,3	GO:0009846	pollen germination	1.6e-136	AT3G07130
			GO:0009845	seed germination		
			GO:0005576	extracellular region		
			GO:0003993	acid phosphatase activity		
			GO:0004722	protein serine/threonine phosphatase activity		
MDP0000172863	Probable inactive receptor kinase	2,3	GO:0006468	protein phosphorylation	1.6e-208	AT1G27190
			GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway		
			GO:0005576	extracellular region		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0005524	ATP binding		
			GO:0016301	kinase activity		
			GO:0004674	protein serine/threonine kinase activity		
MDP0000311339	Heat shock 70 kDa protein	2,3	GO:0010286	heat acclimation	2.4e-59	Hsp70b
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009615	response to virus		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0016020	membrane		
			GO:0005886	plasma membrane		
MDP0000267891	UPF0098 protein MTH_273	2,3	GO:0008150	biological_process	4.4e-54	AT5G01300
			GO:0010264	myo-inositol hexakisphosphate biosynthetic process		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0010162	seed dormancy process		
			GO:0005737	cytoplasm		
			GO:0008429	phosphatidylethanolamine binding		
MDP0000744777	Protein popC	2,2	GO:0006952	defense response	2.2e-62	AT3G25510
MDP0000302297	Somatic embryogenesis receptor kinase 1	2,2	GO:0006355	regulation of transcription, DNA-templated	1.4e-75	B-box domain protein 12, BBX12
			GO:0005634	nucleus		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
			GO:0008270	zinc ion binding		
MDP0000290618	Peroxisome biogenesis factor 10	2,2	GO:0010381	attachment of peroxisome to chloroplast	2.3e-111	PEX10 peroxin 10
			GO:0009793	embryo development ending in seed dormancy		
			GO:0006635	fatty acid beta-oxidation		
			GO:0007031	peroxisome organization		
			GO:0009853	photorespiration		
			GO:0016558	protein import into peroxisome matrix		
			GO:0006513	protein monoubiquitination		

			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0005777	peroxisome		
			GO:0005515	protein binding		
			GO:0004842	ubiquitin-protein ligase activity		
			GO:0008270	zinc ion binding		
MDP0000440811	Cyclin-D3-2	2,1	GO:0008283	cell proliferation	1.2e-19	AT3G50070
			GO:0042023	DNA endoreduplication		
			GO:0000278	mitotic cell cycle		
			GO:0045736	negative regulation of cyclin-dependent protein serine/threonine kinase activity		
			GO:0051726	regulation of cell cycle		
			GO:0042127	regulation of cell proliferation		
			GO:0048316	seed development		
			GO:0010440	stomatal lineage progression		
			GO:0005634	nucleus		
			GO:0004693	cyclin-dependent protein serine/threonine kinase activity		
			GO:0005515	protein binding		
MDP0000122734	Heat shock cognate 70 kDa protein 4	2,1	GO:0006457	protein folding	1.8e-256	AT3G12580
			GO:0016567	protein ubiquitination		
			GO:0009617	response to bacterium		
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009266	response to temperature stimulus		
			GO:0009615	response to virus		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005739	mitochondrion		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
			GO:0031625	ubiquitin protein ligase binding		
MDP0000138306	Polyphosphoinositide phosphatase	2,1	GO:0010413	glucuronoxylan metabolic process	2.4e-264	AT3G43220
			GO:0046855	inositol phosphate dephosphorylation		
			GO:0032957	inositol trisphosphate metabolic process		
			GO:0045492	xylan biosynthetic process		
			GO:0005634	nucleus		
			GO:0004439	phosphatidylinositol-4,5-bisphosphate 5-phosphatase activity		
MDP0000256438	Small heat shock protein, chloroplastic	2,1	GO:0010286	heat acclimation	1.4e-60	AT4G27670
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009507	chloroplast		
MDP0000795157	DnaJ homolog subfamily B member 4	2,1	GO:0006457	protein folding	1.3e-114	AT2G20560
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000164489	18.5 kDa class I heat shock protein	2,1	GO:0010286	heat acclimation	4.5e-41	AT2G29500
			GO:0006457	protein folding		

			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular_function		
MDP0000707429	transferase, transferring glycosyl groups	2,1	GO:0005576	extracellular region	6.0e-105	AT3G18180
MDP0000438504	Phospholipase C 3	2,1	GO:0009395	phospholipid catabolic process	1.2e-88	NPC4 non-specific phospholipase C4
			GO:0005886	plasma membrane		
			GO:0016788	hydrolase activity, acting on ester bonds		
			GO:0004629	phospholipase C activity		
MDP0000314161	Cyclin-A2-3	2,0	GO:0008283	cell proliferation	4.9e-26	CYCLIN A2;3
			GO:0000911	cytokinesis by cell plate formation		
			GO:0042023	DNA endoreduplication		
			GO:0010311	lateral root formation		
			GO:0000226	microtubule cytoskeleton organization		
			GO:0000278	mitotic cell cycle		
			GO:0045736	negative regulation of cyclin-dependent protein serine/threonine kinase activity		
			GO:0000280	nuclear division		
			GO:2000123	positive regulation of stomatal complex development		
			GO:0043248	proteasome assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0051726	regulation of cell cycle		
			GO:0006275	regulation of cell cycle		
			GO:0010389	regulation of G2/M transition of mitotic cell cycle		
			GO:0051510	regulation of unidimensional cell growth		
			GO:0051788	response to misfolded protein		
			GO:0051225	spindle assembly		
			GO:0010440	stomatal lineage progression		
			GO:0005634	nucleus		
			GO:0016538	cyclin-dependent protein serine/threonine kinase regulator activity		
			GO:0005515	protein binding		
MDP0000574524	17.5 kDa class I heat shock protein	2,0	GO:0010286	heat acclimation	6.1e-28	AT2G29500
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000275263	THO complex subunit 4	2,0	GO:0008150	biological process	2.6e-61	AT1G66080
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		

			GO:0003674	molecular function		
MDP0000322220	Heat shock cognate 70 kDa protein 1	2,0	GO:0042742	defense response to bacterium	1.7e-298	HSC70-1 heat shock cognate protein 70-1
			GO:0050832	defense response to fungus		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0010187	negative regulation of seed germination		
			GO:0006457	protein folding		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009651	response to salt stress		
			GO:0009615	response to virus		
			GO:0090332	stomatal closure		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0002020	protease binding		
			GO:0005515	protein binding		
MDP0000932255	Activator of 90 kDa heat shock protein ATPase homolog 1	2,0	GO:0006457	protein folding	6.2e-134	AT3G12050
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
MDP0000160372	REF/SRPP-like protein At1g67360	2,0	GO:0008150	biological process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000205027	Putative uncharacterized protein	2,0	GO:0008150	biological process	9.3e-12	AT2G24395
			GO:0003674	molecular function		
MDP0000207407	18.5 kDa class I heat shock protein	2,0	GO:0010286	heat acclimation	5.3e-42	AT2G29500
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000849143	PPPDE peptidase domain-containing protein 2	2,0	GO:0008150	biological process	2.2e-89	AT3G07090
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000160372	REF/SRPP-like protein At1g67360	2,0	GO:0008150	biological process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000920394	unknown protein	2,0	GO:0008150	biological process	2.7e-11	AT1G32928
			GO:0005575	cellular component		
			GO:0003674	molecular function		

MDP0000929144	unknown protein	2,0	GO:0008150 GO:0009507 GO:0003674	biological process chloroplast molecular function	1.7e-25	AT2G01300
MDP0000271554	HSA32 (HEAT-STRESS-ASSOCIATED 32); catalytic	2,0	GO:0010286 GO:0008152 GO:0010608 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005618 GO:0005634 GO:0004252	heat acclimation metabolic process posttranscriptional regulation of gene expression protein folding response to heat response to high light intensity response to hydrogen peroxide cell wall nucleus serine-type endopeptidase activity	7.3e-114	AT4G21320
MDP0000303430	Heat shock protein 83	2,0	GO:0006457 GO:0048046 GO:0005618 GO:0009570 GO:0005737 GO:0005829 GO:0005794 GO:0005634 GO:0005886 GO:0005774 GO:0005524	protein folding apoplast cell wall chloroplast stroma cytoplasm cytosol Golgi apparatus nucleus plasma membrane vacuolar membrane ATP binding	1.6e-275	AT5G56000
MDP0000446336	Pentatricopeptide repeat-containing protein At4g38150	2,0	GO:0015931 GO:0009507 GO:0015391	nucleobase-containing compound transport chloroplast nucleobase:cation symporter activity	5.1e-85	AT5G03560
MDP0000768256	Hsp70-binding protein 1	2,0	GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0009651 GO:0005737 GO:0005634 GO:0030544	protein folding response to heat response to high light intensity response to hydrogen peroxide response to salt stress cytoplasm nucleus Hsp70 protein binding	2.7e-132	Fes1A
MDP0000125300	Small heat shock protein, chloroplastic	1,9	GO:0006457 GO:0046686 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005739 GO:0003674	protein folding response to cadmium ion response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide mitochondrion molecular function	1.4e-36	AT4G25200
MDP0000304623	Chaperone protein ClpB 2	1,9	GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0009507 GO:0009941 GO:0009570 GO:0005739 GO:0005524 GO:0016887	protein folding response to heat response to high light intensity response to hydrogen peroxide chloroplast chloroplast envelope chloroplast stroma mitochondrion ATP binding ATPase activity	0	AT2G25140
MDP0000508644	unknown protein	1,9	GO:0008150 GO:0030968 GO:0010286 GO:0043069	biological process endoplasmic reticulum unfolded protein response heat acclimation negative regulation of programmed cell death	1.8e-20	AT5G10695

			GO:0006612	protein targeting to membrane		
			GO:0010363	regulation of plant-type hypersensitive response		
			GO:0005576	extracellular region		
			GO:0005739	mitochondrion		
MDP0000285549	Uncharacterized protein At4g08330, chloroplastic	1,9	GO:0008150	biological process	6.0e-48	AT2G17705
			GO:0009507	chloroplast		
			GO:0005886	plasma membrane		
			GO:0003674	molecular function		
MDP0000158047	EXO (EXORDIUM)	1,9	GO:0009741	response to brassinosteroid	8.2e-102	AT4G08950
			GO:0016126	sterol biosynthetic process		
			GO:0005618	cell wall		
			GO:0005576	extracellular region		
			GO:0005794	Golgi apparatus		
			GO:0009505	plant-type cell wall		
			GO:0003674	molecular function		
MDP0000203303	N/A	1,9	GO:0008283	cell proliferation	1.2e-14	APC10 anaphase promoting complex 10
			GO:0042023	DNA endoreduplication		
			GO:0007276	gamete generation		
			GO:0032876	negative regulation of DNA endoreduplication		
			GO:0010087	phloem or xylem histogenesis		
			GO:0043248	proteasome assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0051302	regulation of cell division		
			GO:0032875	regulation of DNA endoreduplication		
			GO:0051510	regulation of unidimensional cell growth		
			GO:0051788	response to misfolded protein		
			GO:0016604	nuclear body		
			GO:0005634	nucleus		
			GO:0005515	protein binding		
MDP0000243895	Heat shock factor protein HSF30	1,9	GO:0034605	cellular response to heat	3.9e-91	AT2G26150
			GO:0071456	cellular response to hypoxia		
			GO:0034620	cellular response to unfolded protein		
			GO:0010286	heat acclimation		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006457	protein folding		
			GO:0010200	response to chitin		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0001666	response to hypoxia		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0005515	protein binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000755970	Heat shock protein 101	1,9	GO:0010286	heat acclimation	0.	AT1G74310
			GO:0045727	positive regulation of translation		
			GO:0006457	protein folding		
			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		

			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
			GO:0005515	protein binding		
MDP0000370315	Arginyl-tRNA synthetase	1,9	GO:0006420	arginyl-tRNA aminoacylation	3.1e-165	AT4G26300
			GO:0048825	cotyledon development		
			GO:0009793	embryo development ending in seed dormancy		
			GO:0048366	leaf development		
			GO:0006164	purine nucleotide biosynthetic process		
			GO:0010564	regulation of cell cycle process		
			GO:0010162	seed dormancy process		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005739	mitochondrion		
			GO:0004814	arginine-tRNA ligase activity		
			GO:0005524	ATP binding		
MDP0000465593	E3 ubiquitin-protein ligase RING1-like	1,9	GO:0005634	nucleus	5.6e-27	AT1G26800
			GO:0008270	zinc ion binding		
MDP0000575835	Uncharacterized calcium-binding protein At1g02270	1,9	GO:0009409	response to cold	7.3e-36	AT1G02270
			GO:0005634	nucleus		
			GO:0005509	calcium ion binding		
			GO:0003824	catalytic activity		
MDP0000807190	ABC transporter B family member 26, chloroplastic	1,9	GO:0015996	chlorophyll catabolic process	5.4e-267	AT1G70610
			GO:0055072	iron ion homeostasis		
			GO:0010039	response to iron ion		
			GO:0000302	response to reactive oxygen species		
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0042626	ATPase activity, coupled to transmembrane movement of substances		
			GO:0005215	transporter activity		
MDP0000214382	25.3 kDa heat shock protein, chloroplastic	1,9	GO:0010286	heat acclimation	2.4e-21	AT4G27670
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009507	chloroplast		
MDP0000226817	EGY3 (ethylene-dependent gravitropism-deficient and yellow-green-like 3)	1,9	GO:0006457	protein folding	3.8e-206	EGY3
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
MDP0000226817	EGY3 (ethylene-dependent gravitropism-deficient and yellow-green-like 3)	1,9	GO:0006457	protein folding	3.8e-206	EGY3
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
MDP0000878773	1-aminocyclopropane-1-carboxylate oxidase homolog 1	1,8	GO:0042538	hyperosmotic salinity response	6.0e-80	AT1G06620
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0005737	cytoplasm		

			GO:0016706	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors		
MDP0000364302		1,8	GO:0008150 GO:0005739 GO:0003674	biological process mitochondrion molecular function	5.1e-18	AT4G32208
MDP0000842179	Granule-bound starch synthase 2, chloroplastic/amyloplastic	1,8	GO:0010021 GO:0019375 GO:0010264 GO:0001666 GO:0009507 GO:0009011 GO:0016757	amylopectin biosynthetic process galactolipid biosynthetic process myo-inositol hexakisphosphate biosynthetic process response to hypoxia chloroplast starch synthase activity transferase activity, transferring glycosyl groups	4.7e-150	SS2 starch synthase 2
MDP0000171695	unknown protein	1,8	GO:0009061 GO:0006979 GO:0005634 GO:0003674	anaerobic respiration response to oxidative stress nucleus molecula function	1.9e-30	AT3G10020
MDP0000181929	Heat shock cognate protein 80	1,8	GO:0071277 GO:0006457 GO:0009408 GO:0005737 GO:0005829 GO:0005794 GO:0016020 GO:0005730 GO:0005886 GO:0005524	cellular response to calcium ion protein folding response to heat cytoplasm cytosol Golgi apparatus membrane nucleolus plasma membrane ATP binding	8.5e-300	AT5G56010
MDP0000254930	PPPDE peptidase domain-containing protein 2	1,8	GO:0008150 GO:0005737 GO:0005634 GO:0003674	biological process cytoplasm nucleus molecular function	8.4e-90	AT3G07090
MDP0000266191	REF/SRPP-like protein At1g67360	1,8	GO:0008150 GO:0005737 GO:0005773	biological process cytoplasm vacuole	3.2e-54	AT1G67360
MDP0000202749	THO complex subunit 4	1,8	GO:0008150 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0003674	biological process protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm cytosol molecular function	4.2e-77	AT1G66080
MDP0000192979	ATMAP70-2 (microtubule-associated proteins 70-2); microtubule binding	1,8	GO:0007010 GO:0005874 GO:0005634 GO:0008017	cytoskeleton organization microtubule nucleus microtubule binding	5.9e-156	MAP70-2 microtubule-associated proteins 70-2
MDP0000446914	Glycogenin-1	1,8	GO:0016051 GO:0006012 GO:0009737 GO:0009409 GO:0009408 GO:0009644 GO:0042542 GO:0006979 GO:0009651 GO:0009414	carbohydrate biosynthetic process galactose metabolic process response to abscisic acid response to cold response to heat response to high light intensity response to hydrogen peroxide response to oxidative stress response to salt stress response to water deprivation	1.1e-148	AT2G47180

			GO:0005575	cellular component		
			GO:0047216	inositol 3-alpha-galactosyltransferase activity		
			GO:0016758	transferase activity, transferring hexosyl groups		
MDP0000536661		1,8	GO:0034968	histone lysine methylation	1.5e-07	PRMT10 protein arginine methyltransferase 10
			GO:0019919	peptidyl-arginine methylation, to asymmetrical-dimethyl arginine		
			GO:0009909	regulation of flower development		
			GO:0010228	vegetative to reproductive phase transition of meristem		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0008469	histone-arginine N-methyltransferase activity		
			GO:0008168	methyltransferase activity		
			GO:0035242	protein-arginine omega-N asymmetric methyltransferase activity		
			GO:0035241	protein-arginine omega-N monomethyltransferase activity		
			GO:0016277	[myelin basic protein]-arginine N-methyltransferase activity		
MDP0000160372	REF/SRPP-like protein At1g67360	1,8	GO:0008150	biological process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000145643	REF/SRPP-like protein At1g67360	1,8	GO:0008150	Biological process	8.4e-54	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000581293	Protein CCA1	1,7	GO:0009851	auxin biosynthetic process	1.5e-52	RVE1 REVEILLE 1
			GO:0009734	auxin-activated signaling pathway		
			GO:0007623	circadian rhythm		
			GO:0048574	long-day photoperiodism, flowering		
			GO:0010600	regulation of auxin biosynthetic process		
			GO:0006355	regulation of transcription, DNA-templated		
			GO:0009737	response to abscisic acid		
			GO:0009733	response to auxin		
			GO:0009723	response to ethylene		
			GO:0009739	response to gibberellin		
			GO:0009753	response to jasmonic acid		
			GO:0009751	response to salicylic acid		
			GO:0009651	response to salt stress		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000209143	Uncharacterized protein R707	1,7	GO:0016051	carbohydrate biosynthetic process	1.2e-131	GolS4 galactinol synthase 4
			GO:0006979	response to oxidative stress		
			GO:0005634	nucleus		
			GO:0016757	transferase activity, transferring glycosyl groups		
			GO:0016758	transferase activity, transferring hexosyl groups		
MDP0000489886	Heat shock factor protein HSF30	1,7	GO:0034605	cellular response to heat	9.0e-86	AT2G26150
			GO:0071456	cellular response to hypoxia		
			GO:0034620	cellular response to unfolded protein		
			GO:0010286	heat acclimation		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006457	protein folding		
			GO:0010200	response to chitin		
			GO:0034976	response to endoplasmic reticulum stress		

			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0001666	response to hypoxia		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0005515	protein binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000849143	PPPDE peptidase domain-containing protein 2	1,7	GO:0008150	biological process	2.2e-89	AT3G07090
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000184300	unknown protein	1,7	GO:0008150	biological process	3.7e-21	AT5G52870
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000205027	Putative uncharacterized protein	1,7	GO:0008150	biological process	9.3e-12	AT2G24395
			GO:0003674	molecular function		
MDP0000119940	Probable protein phosphatase 2C 13	1,7	GO:0004722	protein serine/threonine phosphatase activity	3.4e-100	AT1G48040
MDP0000217508	Heat shock protein 101	1,7	GO:0010286	heat acclimation	0.	HSP101
			GO:0045727	positive regulation of translation		
			GO:0006457	protein folding		
			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
			GO:0005515	protein binding		
MDP0000279217		1,7	GO:0008150	biological process	0.00080	AT5G51160
			GO:0010106	cellular response to iron ion starvation		
			GO:0006826	iron ion transport		
			GO:0015706	nitrate transport		
			GO:0010167	response to nitrate		
			GO:0010043	response to zinc ion		
			GO:0005575	cellular component		
MDP0000549793	ATERDJ3A; oxidoreductase	1,7	GO:0009688	abscisic acid biosynthetic process	4.9e-131	ATERDJ3A
			GO:0034605	cellular response to heat		AT3G08970
			GO:0010286	heat acclimation		
			GO:0009860	pollen tube growth		
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005788	endoplasmic reticulum lumen		
			GO:0009506	plasmodesma		
			GO:0016491	oxidoreductase activity		
MDP0000322510	Elongator complex	1,7	GO:0008283	cell proliferation	3.9e-156	ELO3

	protein 3		GO:0009294 GO:0035265 GO:0051604 GO:0010928 GO:2000025 GO:0010084 GO:0002098 GO:0005829 GO:0033588 GO:0005719 GO:0005634 GO:0004402	DNA mediated transformation organ growth protein maturation regulation of auxin mediated signaling pathway regulation of leaf formation specification of organ axis polarity tRNA wobble uridine modification cytosol Elongator holoenzyme complex nuclear euchromatin nucleus histone acetyltransferase activity		ELONGATA 3
MDP0000168932	Cysteine proteinase RD21a	1,7	GO:0005783 GO:0005576 GO:0005773 GO:0008234	endoplasmic reticulum extracellular region vacuole cysteine-type peptidase activity	4.1e-115	XBCP3 xylem bark cysteine peptidase 3
MDP0000275837	Pentatricopeptide repeat-containing protein At5g02860	1,7	GO:0008150 GO:0005739	biological process mitochondrion	1.8e-131	AT5G02860
MDP0000243895	Heat shock factor protein HSF30	1,7	GO:0034605 GO:0071456 GO:0034620 GO:0010286 GO:0045893 GO:0006457 GO:0010200 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0001666 GO:0009407 GO:0009507 GO:0005634 GO:0003677 GO:0005515 GO:0003700	cellular response to heat cellular response to hypoxi cellular response to unfolded protein heat acclimation positive regulation of transcription, DNA-templated protein folding response to chitin response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide response to hypoxia toxin catabolic process chloroplast nucleus DNA binding protein binding sequence-specific DNA binding transcription factor activity	3.9e-91	AT2G26150
MDP0000614608	Probable VAMP-like protein	1,7	GO:0003674	molecular function	4.0e-44	AT5G52990
MDP0000186561	Peptidyl-prolyl cis-trans isomerase cyp5	1,7	GO:0000398 GO:0006457 GO:0005634 GO:0003755	mRNA splicing, via spliceosome protein folding nucleus peptidyl-prolyl cis-trans isomerase activity	4.4e-102	AT4G32420
MDP0000152242	Triosephosphate isomerase, cytosolic	1,7	GO:0009060 GO:0044262 GO:0006094 GO:0006096 GO:0019344 GO:0007030 GO:0006972 GO:0008152 GO:0006098 GO:0009853 GO:0080129 GO:0043161	aerobic respiration cellular carbohydrate metabolic process gluconeogenesis glycolysis cysteine biosynthetic process Golgi organization hyperosmotic response metabolic process pentose-phosphate shunt photorespiration proteasome core complex assembly proteasome-mediated ubiquitin-dependent protein catabolic process	6.8e-102	AT3G55440

			GO:0046686	response to cadmium ion		
			GO:0051788	response to misfolded protein		
			GO:0009651	response to salt stress		
			GO:0009266	response to temperature stimulus		
			GO:0010043	response to zinc ion		
			GO:0006511	ubiquitin-dependent protein catabolic process		
			GO:0006833	water transport		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005739	mitochondrion		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0005773	vacuole		
			GO:0005507	copper ion binding		
			GO:0004807	triose-phosphate isomerase activity		
MDP0000264662	Chaperone protein ClpB 2	1,7	GO:0009658	chloroplast organization	0.	AT5G15450
			GO:0009408	response to heat		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0009532	plastid stroma		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		

Downregulated genes						
MDP0000240840	Ethylene-responsive transcription factor 5	0,5	GO:0009873 GO:0035556 GO:0045893 GO:0006355 GO:0002679 GO:0010200 GO:0009409 GO:0005634 GO:0003677 GO:0003700	ethylene activated signaling pathway intracellular signal transduction positive regulation of transcription, DNA-templated regulation of transcription, DNA-templated respiratory burst involved in defense response response to chitin response to cold nucleus DNA binding sequence-specific DNA binding transcription factor activity	3.6e-39	ERF5
MDP0000383777	Amino acid permease 3	0,5	GO:0043090 GO:0006865 GO:0015696 GO:0006820 GO:0015802 GO:0006888 GO:0043069 GO:0006862 GO:0006612 GO:0043269 GO:0010363 GO:0016020 GO:0005886 GO:0015171	amino acid import amino acid transport ammonium transport anion transport basic amino acid transport ER to Golgi vesicle-mediated transport negative regulation of programmed cell death nucleotide transport protein targeting to membrane regulation of ion transport regulation of plant-type hypersensitive response membrane plasma membrane amino acid transmembrane transporter activity	1.1e-195	AAP3 amino acid permease 3
MDP0000323212	Auxin-repressed 12.5 kDa protein	0,5	GO:0005634	nucleus	2.0e-24	DRM2
MDP0000720785	Expression of the gene is downregulated in the presence of paraquat, an inducer of photoxidative stress.	0,5	GO:0006979 GO:0005634 GO:0003674	response to oxidative stress nucleus molecular function	5.8e-16	AT3G20340
MDP0000334850	wound-responsive protein-related	0,5	GO:0008150 GO:0005634 GO:0003674	biological process nucleus molecular function	5.2e-14	AT4G28240
MDP0000184167	Nucleolar GTP-binding protein 1	0,5	GO:0005634	nucleus	4.9e-78	PDE318
MDP0000906115	Flavonol synthase/flavanone 3-hydroxylase	0,5	GO:0009058 GO:0005737 GO:0016706	biosynthetic process cytoplasm oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors	6.0e-46	AT2G36690
MDP0000257621	EB1C from Arabidopsis thalina	0,5	GO:0030865 GO:0016572 GO:0000278 GO:0009652 GO:0010228 GO:0005618 GO:0005737 GO:0005730	cortical cytoskeleton organization histone phosphorylation mitotic cell cycle thigmotropism vegetative to reproductive phase transition of meristem cell wall cytoplasm nucleolus	5.1e-16	EB1C

			GO:0005634 GO:0009524 GO:0005819 GO:0008017	nucleus phragmoplast spindle microtubule binding		
MDP0000939633	AP2/ERF and B3 domain-containing transcription repressor TEM1	0,5	GO:0030003 GO:0070838 GO:0009873 GO:0048573 GO:0005634 GO:0003677 GO:0003700	cellular cation homeostasis divalent metal ion transport ethylene-activated signaling pathway photoperiodism, flowering nucleus DNA binding sequence-specific DNA binding transcription factor activity	3.7e-95	TEM1 TEMPRANILLO 1
MDP0000279037	unknown protein	0,5	GO:0008150 GO:0003674	biological process molecular function	1.4e-142	AT1G63300
MDP0000939989	Enolase	0,4	GO:0006094 GO:0006096 GO:0006098 GO:0009853 GO:0009737 GO:0046686 GO:0009409 GO:0009416 GO:0009651 GO:0048046 GO:0009507 GO:0005737 GO:0005829 GO:0016020 GO:0005740 GO:0005739 GO:0005634 GO:0005886 GO:0009506 GO:0005507 GO:0003677 GO:0004634	gluconeogenesis glycolysis pentose-phosphate shunt photorespiration response to abscisic acid response to cadmium ion response to cold response to light stimulus response to salt stress apoplast chloroplast cytoplasm cytosol membrane mitochondrial envelope mitochondrion nucleus plasma membrane plasmodesma copper ion binding DNA binding phosphopyruvate hydratase activity	1.3e-198	AT2G36530

Table 5

Transcripts differentially expressed in HT fruit compare to the control after 1 hour from the treatment.

MDP	Description	Ratio	GO code	GO terms	p value	*Arabidopsis* protein
Upregulated genes						
MDP0000543224	Probable pyridoxal biosynthesis protein PDX1.2	4,8	GO:0006457	protein folding	1.1e-111	PDX1.2, pyridoxine biosynthesis 1.2
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0042819	vitamin B6 biosynthetic process		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005515	protein binding		
			GO:0046982	protein heterodimerization activity		
MDP0000657396	Protein FAM32A	4,2	GO:0008150	biological process	1.8e-18	AT1G16810
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000209621	unknown protein	3,8	GO:0008150	biological process	7.7e-41	AT5G47830
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	3,7	GO:0016049	cell growth	2.5e-167	EYE
			GO:0009793	embryo development ending in seed dormancy		
			GO:0007030	Golgi organization		
			GO:0048507	meristem development		
			GO:0009933	meristem structural organization		
			GO:0045053	protein retention in Golgi apparatus		
			GO:0010016	shoot system morphogenesis		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0003674	molecular function		
MDP0000171695	unknown protein	3,6	GO:0009061	anaerobic respiration	1.9e-30	AT3G10020
			GO:0006979	response to oxidative stress		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000684170	Heat shock cognate 70 kDa protein 4	3,5	GO:0010286	heat acclimation	7.2e-217	heat-shock protein 70T-2
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000311339	Heat shock 70 kDa protein	3,4	GO:0010286	heat acclimation	2.4e-59	Hsp70b
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009615	response to virus		

			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0016020	membrane		
			GO:0005886	plasma membrane		
MDP0000290546	DnaJ homolog subfamily B member 13	3,3	GO:0006457	protein folding	4.1e-120	
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000254930	PPPDE peptidase domain-containing protein 2	3,2	GO:0008150	biological process	8.4e-90	AT3G07090
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000121482	Probable LRR receptor-like serine/threonine-protein kinase	3,2	GO:0009718	anthocyanin-containing compound biosynthetic process	5.3e-131	AT3G03770
			GO:0006468	protein phosphorylation		
			GO:0009744	response to sucrose		
			GO:0010224	response to UV-B		
			GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway		
			GO:0005886	plasma membrane		
			GO:0005524	ATP binding		
			GO:0004672	protein kinase activity		
			GO:0004674	protein serine/threonine kinase activity		
MDP0000125300	Small heat shock protein, chloroplastic	3,2	GO:0006457	protein folding	1.4e-36	HSP23.6-MITO AT4G25200
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000271554	HSA32	3,1	GO:0010286	heat acclimation	7.3e-114	AT4G21320
			GO:0008152	metabolic process		
			GO:0010608	posttranscriptional regulation of gene expression		
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005618	cell wall		
			GO:0005634	nucleus		
			GO:0004252	serine-type endopeptidase activity		
MDP0000302297	Somatic embryogenesis receptor kinase 1	3,1	GO:0006355	regulation of transcription, DNA-templated	1.4e-75	B-box domain protein 12, BBX12
			GO:0005634	nucleus		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
			GO:0008270	zinc ion binding		
MDP0000318167	Zinc finger protein CONSTANS-LIKE 4	3,1	GO:0008150	biological process	1.4e-78	AT1G04500
			GO:0009827	plant-type cell wall modification		
			GO:0009860	pollen tube growth		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000205027	Putative	3,1	GO:0008150	biological process	9.3e-12	AT2G24395

	uncharacterized protein		GO:0003674	molecular function		
MDP0000720039	Polyubiquitin	3,1	GO:0006464 GO:0042787 GO:0006511	cellular protein modification process protein ubiquitination involved in ubiquitin-dependent protein catabolic process ubiquitin-dependent protein catabolic process	1.4e-135	AT5G20620
MDP0000508644	unknown protein	3,1	GO:0008150 GO:0030968 GO:0010286 GO:0043069 GO:0006612 GO:0010363 GO:0005576 GO:0005739	biological process endoplasmic reticulum unfolded protein response heat acclimation negative regulation of programmed cell death protein targeting to membrane regulation of plant-type hypersensitive response extracellular region mitochondrion	1.8e-20	AT5G10695
MDP0000145643	REF/SRPP-like protein At1g67360	3,0	GO:0008150 GO:0005737 GO:0005773	biological process cytoplasm vacuole	8.4e-54	AT1G67360
MDP0000549793	oxidoreductase	3,0	GO:0009688 GO:0034605 GO:0010286 GO:0009860 GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005788 GO:0009506 GO:0016491	abscisic acid biosynthetic process cellular response to heat heat acclimation pollen tube growth protein folding response to heat response to high light intensity response to hydrogen peroxide endoplasmic reticulum lumen plasmodesma oxidoreductase activity	4.9e-131	AT3G08970
MDP0000849143	PPPDE peptidase domain-containing protein 2	2,9	GO:0008150 GO:0005737 GO:0005634 GO:0003674	biological process cytoplasm nucleus molecular function	2.2e-89	AT3G07090
MDP0000164489	18.5 kDa class I heat shock protein	2,9	GO:0010286 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0006979 GO:0005737 GO:0003674	heat acclimation protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide response to oxidative stress cytoplasm molecular function	4.5e-41	AT2G29500
MDP0000207407	18.5 kDa class I heat shock protein	2,9	GO:0010286 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0006979 GO:0005737 GO:0003674	heat acclimation protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide response to oxidative stress cytoplasm molecular function	5.3e-42	AT2G29500
MDP0000303015	Heat shock protein 101	2,9	GO:0010286 GO:0045727 GO:0006457	heat acclimation positive regulation of translation protein folding	0	AT1G74310

			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
MDP0000254930	PPPDE peptidase domain-containing protein 2	2,9	GO:0008150	biological process	8.4e-90	AT3G07090
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000755113	Probable glutathione S-transferase	2,9	GO:0016036	cellular response to phosphate starvation	1.4e-85	AT1G78380
			GO:0042631	cellular response to water deprivation		
			GO:0006635	fatty acid beta-oxidation		
			GO:0019375	galactolipid biosynthetic process		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0080129	proteasome core complex assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0046686	response to cadmium ion		
			GO:0010583	response to cyclopentenone		
			GO:0051788	response to misfolded protein		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0043295	glutathione binding		
			GO:0004364	glutathione transferase activity		
MDP0000878773	1-aminocyclopropane-1-carboxylate oxidase homolog 1	2,8	GO:0042538	hyperosmotic salinity response	6.0e-80	AT1G06620
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0005737	cytoplasm		
			GO:0016706	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors		
MDP0000707429	transferase, transferring glycosyl groups	2,8	GO:0005576	extracellular region	6.0e-105	AT3G18180
MDP0000290618	Peroxisome biogenesis factor 10	2,8	GO:0010381	attachment of peroxisome to chloroplast	2.3e-111	PEX10 peroxin 10
			GO:0009793	embryo development ending in seed dormancy		
			GO:0006635	fatty acid beta-oxidation		
			GO:0007031	peroxisome organization		
			GO:0009853	photorespiration		
			GO:0016558	protein import into peroxisome matrix		
			GO:0006513	protein monoubiquitination		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0005777	peroxisome		

			GO:0005515	protein binding		
			GO:0004842	ubiquitin-protein ligase activity		
			GO:0008270	zinc ion binding		
MDP0000744777	Protein popC	2,8	GO:0006952	defense response	2.2e-62	AT3G25510
MDP0000466683	Glycogenin-1	2,8	GO:0016051	carbohydrate biosynthetic process	1.5e-148	AT2G47180
			GO:0006012	galactose metabolic process		
			GO:0009737	response to abscisic acid		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxid		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009414	response to water deprivation		
			GO:0005575	cellular_component		
			GO:0047216	inositol 3-alpha-galactosyltransferase activity		
			GO:0016758	transferase activity, transferring hexosyl groups		
MDP0000247652	17.3 kDa class I heat shock protein	2,8	GO:0010286	heat acclimation	1.1e-41	HSP18.2
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000243895	Heat shock factor protein HSF30	2,7	GO:0034605	cellular response to heat	3.9e-91	AT2G26150
			GO:0071456	cellular response to hypoxia		
			GO:0034620	cellular response to unfolded protein		
			GO:0010286	heat acclimation		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006457	protein folding		
			GO:0010200	response to chitin		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0001666	response to hypoxia		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0005515	protein binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000755970	HSP 101	2,7	GO:0010286	heat acclimation	0.	AT1G74310
			GO:0045727	positive regulation of translation		
			GO:0006457	protein folding		
			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		

			GO:0005515	protein binding		
MDP0000438504	Phospholipase C 3	2,7	GO:0009395	phospholipid catabolic process	1.2e-88	NPC4 non-specific phospholipase C4
			GO:0005886	plasma membrane		
			GO:0016788	hydrolase activity, acting on ester bonds		
			GO:0004629	phospholipase C activity		
MDP0000291831	17.9 kDa class II heat shock protein	2,6	GO:0006457	protein folding	1.5e-36	HSP17.6II
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000225534	Purple acid phosphatase 23	2,6	GO:0009846	pollen germination	1.6e-136	AT3G07130
			GO:0009845	seed germination		
			GO:0005576	extracellular region		
			GO:0003993	acid phosphatase activity		
			GO:0004722	protein serine/threonine phosphatase activity		
MDP0000768256	Hsp70-binding protein 1	2,6	GO:0006457	protein folding	2.7e-132	Fes1A
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009651	response to salt stress		
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0030544	Hsp70 protein binding		
MDP0000122734	Heat shock cognate 70 kDa protein 4	2,6	GO:0006457	protein folding	1.8e-256	AT3G12580
			GO:0016567	protein ubiquitination		
			GO:0009617	response to bacterium		
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009266	response to temperature stimulus		
			GO:0009615	response to virus		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005739	mitochondrion		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
			GO:0031625	ubiquitin protein ligase binding		
MDP0000446914	Glycogenin-1	2,6	GO:0016051	carbohydrate biosynthetic process	1.1e-148	AT2G47180
			GO:0006012	galactose metabolic process		
			GO:0009737	response to abscisic acid		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009414	response to water deprivation		
			GO:0005575	cellular_component		
			GO:0047216	inositol 3-alpha-galactosyltransferase activity		

			GO:0016758	transferase activity		
MDP0000422652	Heat shock protein STI	2,6	GO:0009165	nucleotide biosynthetic process	4.5e-229	Hop2
			GO:0046686	response to cadmium ion		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
MDP0000700383	17.9 kDa class II heat shock protein	2,6	GO:0006457	protein folding	8.8e-35	HSP17.6II
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000303430	Heat shock protein 83	2,6	GO:0006457	protein folding	1.6e-275	AT5G56000
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
MDP0000249171	unknown protein	2,6	GO:0008150	biological_process	4.2e-25	AT5G07330
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0003674	molecular_function		
MDP0000217508	Heat shock protein 101	2,6	GO:0010286	heat acclimation	0	AT1G74310
			GO:0045727	positive regulation of translation		
			GO:0006457	protein folding		
			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
			GO:0005515	protein binding		
MDP0000795157	DnaJ homolog subfamily B member 4	2,6	GO:0006457	protein folding	1.3e-114	AT2G20560
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000196824	Probably inactive leucine-rich repeat receptor-like protein kinase	2,5	GO:0006952	defense response	7.2e-70	AT3G11080
			GO:0007165	signal transduction		
			GO:0016301	kinase activity		
MDP0000153978	ATBAG5	2,5	GO:0050832	defense response to fungus	2.9e-12	BAG6

	(ARABIDOPSIS THALIANA BCL-2-ASSOCIATED ATHANOGENE 5); protein binding		GO:0010286 GO:0012502 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0010228 GO:0005634 GO:0009506 GO:0005516	heat acclimation induction of programmed cell death protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide vegetative to reproductive phase transition of meristem nucleus plasmodesma calmodulin binding		
MDP0000314161	Cyclin-A2-3	2,5	GO:0008283 GO:0000911 GO:0042023 GO:0010311 GO:0000226 GO:0000278 GO:0045736 GO:0000280 GO:2000123 GO:0043248 GO:0043161 GO:0051726 GO:0006275 GO:0010389 GO:0051510 GO:0051788 GO:0051225 GO:0010440 GO:0005634 GO:0016538 GO:0005515	cell proliferation cytokinesis by cell plate formation DNA endoreduplication lateral root formation microtubule cytoskeleton organization mitotic cell cycle negative regulation of cyclin-dependent protein serine/threonine kinase activity nuclear division positive regulation of stomatal complex development proteasome assembly proteasome-mediated ubiquitin-dependent protein catabolic process regulation of cell cycle regulation of cell cycle regulation of G2/M transition of mitotic cell cycle regulation of unidimensional cell growth response to misfolded protein spindle assembly stomatal lineage progression nucleus cyclin-dependent protein serine/threonine kinase regulator activity protein binding	4.9e-26	CYCLIN A2;3
MDP0000574524	17.5 kDa class I heat shock protein	2,5	GO:0010286 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0006979 GO:0005737 GO:0003674	heat acclimation protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide response to oxidative stress cytoplasm molecular_function	6.1e-28	AT2G29500
MDP0000137929	Ammonium transporter 3 member 1	2,5	GO:0009738 GO:0043090 GO:0015696 GO:0006820 GO:0015802 GO:0007154	abscisic acid-activated signaling pathway amino acid import ammonium transport anion transport basic amino acid transport cell communication	4.6e-190	AMT2 ammonium transporter 2

			GO:0006995	cellular response to nitrogen starvation		
			GO:0042742	defense response to bacterium		
			GO:0050832	defense response to fungus		
			GO:0009595	detection of biotic stimulus		
			GO:0009581	detection of external stimulus		
			GO:0030968	endoplasmic reticulum unfolded protein response		
			GO:0006888	ER to Golgi vesicle-mediated transport		
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0000165	MAPK cascade		
			GO:0031348	negative regulation of defense response		
			GO:0043069	negative regulation of programmed cell death		
			GO:0006862	nucleotide transport		
			GO:0015695	organic cation transport		
			GO:0006612	protein targeting to membrane		
			GO:0031347	regulation of defense response		
			GO:0010310	regulation of hydrogen peroxide metabolic process		
			GO:0043269	regulation of ion transport		
			GO:0043900	regulation of multi-organism process		
			GO:0010363	regulation of plant-type hypersensitive response		
			GO:0010200	response to chitin		
			GO:0009409	response to cold		
			GO:0009750	response to fructose		
			GO:0009749	response to glucose		
			GO:0002237	response to molecule of bacterial origin		
			GO:0009624	response to nematode		
			GO:0051707	response to other organism		
			GO:0009744	response to sucrose		
			GO:0009697	salicylic acid biosynthetic process		
			GO:0009863	salicylic acid mediated signaling pathway		
			GO:0009627	systemic acquired resistance		
			GO:0009862	systemic acquired resistance, salicylic acid mediated signaling pathway		
			GO:0005737	cytoplasm		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0008519	ammonium transmembrane transporter activity		
			GO:0015398	high affinity secondary active ammonium transmembrane transporter activity		
MDP0000160372	REF/SRPP-like protein	2,5	GO:0008150	biological_process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000768256	Hsp70-binding protein 1	2,5	GO:0006457	protein folding	2.7e-132	Fes1A
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009651	response to salt stress		
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0030544	Hsp70 protein binding		
MDP0000370184	Putative	2,5	GO:0008150	biological_process	0.00032	AT4G25170

	uncharacterized protein		GO:0003674	molecular_function		
MDP0000130123	NAC domain-containing protein 29	2,5	GO:0010150 GO:0007275 GO:0045892 GO:0009963 GO:0006612 GO:0010363 GO:0009737 GO:0009741 GO:0009651 GO:0010089 GO:0005634 GO:0005515	leaf senescence multicellular organismal development negative regulation of transcription, DNA-templated positive regulation of flavonoid biosynthetic process protein targeting to membrane regulation of plant-type hypersensitive response response to abscisic acid response to brassinosteroid response to salt stress xylem development nucleus protein binding	1.1e-69	NAC083 NAC domain containing protein 83
MDP0000203303	N/A	2,4	GO:0008283 GO:0042023 GO:0007276 GO:0032876 GO:0010087 GO:0043248 GO:0043161 GO:0051302 GO:0032875 GO:0051510 GO:0051788 GO:0016604 GO:0005634 GO:0005515	cell proliferation DNA endoreduplication gamete generation negative regulation of DNA endoreduplication phloem or xylem histogenesis proteasome assembly proteasome-mediated ubiquitin-dependent protein catabolic process regulation of cell division regulation of DNA endoreduplication regulation of unidimensional cell growth response to misfolded protein nuclear body nucleus protein binding	1.2e-14	APC10 anaphase promoting complex 10
MDP0000275263	THO complex subunit 4	2,4	GO:0008150 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0003674	biological_process protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm cytosol molecular_function	2.6e-61	AT1G66080
MDP0000313388	Isoamylase 1, chloroplastic	2,4	GO:0010021 GO:0005975 GO:0000023 GO:0043085 GO:0019252 GO:0009507 GO:0010368 GO:0004556 GO:0019156	amylopectin biosynthetic process carbohydrate metabolic process maltose metabolic process positive regulation of catalytic activity starch biosynthetic process chloroplast chloroplast isoamylase complex alpha-amylase activity isoamylase activity	4.9e-271	AT2G39930
MDP0000217855	DnaJ homolog subfamily B member 6	2,4	GO:0008150 GO:0006487 GO:0005634 GO:0005516	biological_process protein N-linked glycosylation nucleus calmodulin binding	2.6e-69	AT5G57580
MDP0000849143	PPPDE peptidase	2,4	GO:0008150	biological_process	2.2e-89	AT3G07090

	domain-containing protein 2		GO:0005737 GO:0005634 GO:0003674	cytoplasm nucleus molecular_function		
MDP0000236662	WD repeat-containing protein 55 homolog	2,4	GO:0008150 GO:0009507 GO:0008234	biological_process chloroplast cysteine-type peptidase activity	1.0e-77	AT3G57810
MDP0000154937	Sn1-specific diacylglycerol lipase beta	2,4	GO:0006629 GO:0005886 GO:0004806	lipid metabolic process plasma membrane triglyceride lipase activity	3.4e-166	AT2G42450
MDP0000303430	Heat shock protein 83	2,4	GO:0006457 GO:0048046 GO:0005618 GO:0009570 GO:0005737 GO:0005829 GO:0005794 GO:0005634 GO:0005886 GO:0005774 GO:0005524	protein folding apoplast cell wall chloroplast stroma cytoplasm cytosol Golgi apparatus nucleus plasma membrane vacuolar membrane ATP binding	1.6e-275	AT5G56000
MDP0000152564	17.4 kDa class III heat shock protein	2,4	GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005737 GO:0003674	protein folding response to heat response to high light intensity response to hydrogen peroxide cytoplasm molecular function	8.4e-29	AT1G54050
MDP0000160372	REF/SRPP-like protein	2,4	GO:0008150 GO:0005737 GO:0005773	biological process cytoplasm vacuole	1.2e-53	AT1G67360
MDP0000126528	F-box protein GID2	2,4	GO:0048444 GO:0009740 GO:0042538 GO:0009867 GO:0009939 GO:0010325 GO:0009737 GO:0009723 GO:0009863 GO:0010162 GO:0009845 GO:0005634 GO:0019005 GO:0005515	floral organ morphogenesis gibberellic acid mediated signaling pathway hyperosmotic salinity response jasmonic acid mediated signaling pathway positive regulation of gibberellic acid mediated signaling pathway raffinose family oligosaccharide biosynthetic process response to abscisic acid response to ethylene salicylic acid mediated signaling pathway seed dormancy process seed germination nucleus SCF ubiquitin ligase complex protein binding	1.0e-42	SLY1 SLEEPY1
MDP0000238655	Uncharacterized RNA-binding protein C25G10.01	2,4	GO:0043484 GO:0009644 GO:0008380 GO:0003723	regulation of RNA splicing response to high light intensity RNA splicing RNA binding	1.2e-35	AT1G07350
MDP0000266191	REF/SRPP-like protein	2,4	GO:0008150 GO:0005737 GO:0005773	biological process cytoplasm vacuole	3.2e-54	AT1G67360
MDP0000203813	RCC1 domain-containing protein 1	2,4	GO:0008150 GO:0005737	biological process cytoplasm	1.2e-156	AT3G55580
MDP0000243895	Heat shock factor protein HSF30	2,4	GO:0034605 GO:0071456 GO:0034620	cellular response to heat cellular response to hypoxia cellular response to unfolded	3.9e-91	AT2G26150

				protein		
			GO:0010286	heat acclimation		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006457	protein folding		
			GO:0010200	response to chitin		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0001666	response to hypoxia		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0005515	protein binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000160372	REF/SRPP-like protein At1g67360	2,4	GO:0008150	biological process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000122734	Heat shock cognate 70 kDa protein 4	2,3	GO:0006457	protein folding	1.8e-256	AT3G12580
			GO:0016567	protein ubiquitination		
			GO:0009617	response to bacterium		
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009266	response to temperature stimulus		
			GO:0009615	response to virus		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005739	mitochondrion		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
			GO:0031625	ubiquitin protein ligase binding		
MDP0000563240	Polyadenylate-binding protein-interacting protein 2	2,3	GO:0006096	glycolysis	4.2e-16	AT2G41430
			GO:0007030	Golgi organization		
			GO:0042744	hydrogen peroxide catabolic proces		
			GO:0006972	hyperosmotic response		
			GO:0032880	regulation of protein localization		
			GO:0009646	response to absence of light		
			GO:0009617	response to bacterium		
			GO:0046686	response to cadmium ion		
			GO:0009644	response to high light intensity		
			GO:0009651	response to salt stress		
			GO:0009266	response to temperature stimulus		
			GO:0009414	response to water deprivation		
			GO:0006833	water transport		
			GO:0005737	cytoplasm		
			GO:0005515	protein binding		
MDP0000214382	25.3 kDa heat	2,3	GO:0010286	heat acclimation	2.4e-21	HSP21

	shock protein, chloroplastic		GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0009507	protein folding response to heat response to high light intensity response to hydrogen peroxide chloroplast		AT4G27670
MDP0000150990	Random slug protein 5	2,3	GO:0008150 GO:0005739 GO:0003674	biological_process mitochondrion molecular_function	1.2e-92	AT3G22410
MDP0000236662	WD repeat-containing protein 55 homolog	2,3	GO:0008150 GO:0009507 GO:0008234	biological_process chloroplast cysteine-type peptidase activity	1.1e-77	AT3G57810
MDP0000446336	Pentatricopeptide repeat-containing protein	2,3	GO:0015931 GO:0009507 GO:0015391	nucleobase-containing compound transport chloroplast nucleobase:cation symporter activity	5.1e-85	AT5G03560
MDP0000186670		2,3	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm	1.5e-10	AT1G53540
MDP0000235765	Chaperonin CPN60-2, mitochondrial	2,3	GO:0051131 GO:0007005 GO:0006457 GO:0006626 GO:0046686 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005829 GO:0022626 GO:0005759 GO:0005739 GO:0005774 GO:0005524 GO:0005507	chaperone-mediated protein complex assembly mitochondrion organization protein folding protein targeting to mitochondrion response to cadmium ion response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytosol cytosolic ribosome mitochondrial matrix mitochondrion vacuolar membrane ATP binding copper ion binding	2.1e-253	HSP60
MDP0000872073	Putative uncharacterized protein	2,3	GO:0008150 GO:0005575 GO:0003674	biological process cellular component molecular function	0.0093	orf19.4959
MDP0000465593	E3 ubiquitin-protein ligase RING1-like	2,2	GO:0005634 GO:0008270	nucleus zinc ion binding	5.6e-27	AT1G26800
MDP0000172863	Probable inactive receptor kinase At1g27190	2,2	GO:0006468 GO:0007169 GO:0005576 GO:0005634 GO:0005886 GO:0005524 GO:0016301 GO:0004674	protein phosphorylation transmembrane receptor protein tyrosine kinase signaling pathway extracellular region nucleus plasma membrane ATP binding kinase activity protein serine/threonine kinase activity	1.6e-208	AT1G27190
MDP0000238655	Uncharacterized RNA-binding protein C25G10.01	2,2	GO:0043484 GO:0009644 GO:0008380 GO:0003723	regulation of RNA splicing response to high light intensity RNA splicing RNA binding	1.2e-35	AT1G07350

MDP0000295085	Ubiquitin fusion degradation protein 1 homolog	2,2	GO:0005634	nucleus	1.5e-190	AT4G15420
MDP0000279776	Putative pentatricopeptide repeat-containing protein At2g02150	2,2	GO:0008150 GO:0005739 GO:0003674	biological process mitochondrion molecular function	2.9e-94	AT2G01740
MDP0000930889	Polyubiquitin	2,2	GO:0007568 GO:0006464 GO:0009751	aging cellular protein modification process response to salicylic acid	1.8e-154	AT4G05320
MDP0000190008	10 kDa chaperonin	2,2	GO:0005737 GO:0005739	cytoplasm mitochondrion	6.8e-38	AT1G23100
MDP0000456824	Chaperone protein dnaJ 11, chloroplastic	2,1	GO:0006457	protein folding	2.1e-23	AT3G13310
MDP0000205027	Putative uncharacterized protein	2,1	GO:0008150 GO:0003674	biological_process molecular_function	9.3e-12	AT2G24395
MDP0000614851	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	2,1	GO:0009658 GO:0009790 GO:0009793 GO:0016226 GO:0048481 GO:0006457 GO:0048046 GO:0009507 GO:0009941 GO:0009570 GO:0005829 GO:0022626 GO:0016020 GO:0005739 GO:0009579	chloroplast organization embryo development embryo development ending in seed dormancy iron-sulfur cluster assembly ovule development protein folding apoplast chloroplast chloroplast envelope chloroplast stroma cytosol cytosolic ribosome membrane mitochondrion thylakoid	1.4e-238	CPN60A chaperonin-60alpha
MDP0000527802	Heat shock factor protein HSF24	2,1	GO:0030968 GO:0006984 GO:0042538 GO:0045892 GO:0009737 GO:0010583 GO:0034976 GO:0009408 GO:0009414 GO:0009407 GO:0005634 GO:0003677 GO:0003700	endoplasmic reticulum unfolded protein response ER-nucleus signaling pathway hyperosmotic salinity response negative regulation of transcription, DNA-templated response to abscisic acid response to cyclopentenone response to endoplasmic reticulum stress response to heat response to water deprivation toxin catabolic process nucleus DNA binding sequence-specific DNA binding transcription factor activity	2.2e-62	AT4G36990
MDP0000184300	unknown protein	2,1	GO:0008150 GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005634 GO:0003674	biological_process protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide nucleus molecular_function	3.7e-21	AT5G52870
MDP0000685137	20 kDa chaperonin,	2,1	GO:0009658	chloroplast organization	5.9e-94	CPN20

	chloroplastic		GO:0019344	cysteine biosynthetic process		chaperonin 20
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0019288	isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway		
			GO:0048481	ovule development		
			GO:1901671	positive regulation of superoxide dismutase activity		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0009651	response to salt stress		
			GO:0048046	apoplast		
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0009535	chloroplast thylakoid membrane		
			GO:0005829	cytosol		
			GO:0005739	mitochondrion		
			GO:0009536	plastid		
			GO:0009579	thylakoid		
			GO:0005516	calmodulin binding		
			GO:0005507	copper ion binding		
			GO:0005515	protein binding		
MDP0000322220	Heat shock cognate 70 kDa protein 1	2,1	GO:0042742	defense response to bacterium	1.7e-298	HSC70-1
			GO:0050832	defense response to fungus		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0010187	negative regulation of seed germination		
			GO:0006457	protein folding		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009651	response to salt stress		
			GO:0009615	response to virus		
			GO:0090332	stomatal closure		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0002020	protease binding		
			GO:0005515	protein binding		
MDP0000161691	Heat shock protein STI	2,1	GO:0009165	nucleotide biosynthetic process	2.1e-213	Hop2
			GO:0046686	response to cadmium ion		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		

MDP0000710281	N/A	2,1	GO:0006508 GO:0009737 GO:0009414 GO:0005783 GO:0005576 GO:0070001	proteolysis response to abscisic acid response to water deprivation endoplasmic reticulum extracellular region aspartic-type peptidase activity	4.4e-19	ASPG1 ASPARTIC PROTEASE IN GUARD CELL 1
MDP0000152242	Triosephosphate isomerase, cytosolic	2,1	GO:0009060 GO:0044262 GO:0006094 GO:0006096 GO:0019344 GO:0007030 GO:0006972 GO:0008152 GO:0006098 GO:0009853 GO:0080129 GO:0043161 GO:0046686 GO:0051788 GO:0009651 GO:0009266 GO:0010043 GO:0006511 GO:0006833 GO:0048046 GO:0005618 GO:0009507 GO:0009570 GO:0005737 GO:0005829 GO:0005794 GO:0005739 GO:0005886 GO:0009506 GO:0005774 GO:0005773 GO:0005507 GO:0004807	aerobic respiration cellular carbohydrate metabolic process gluconeogenesis glycolysis cysteine biosynthetic process Golgi organization hyperosmotic response metabolic process pentose-phosphate shunt photorespiration proteasome core complex assembly proteasome-mediated ubiquitin-dependent protein catabolic process response to cadmium ion response to misfolded protein response to salt stress response to temperature stimulus response to zinc ion ubiquitin-dependent protein catabolic process water transport apoplast cell wall chloroplast chloroplast stroma cytoplasm cytosol Golgi apparatus mitochondrion plasma membrane plasmodesma vacuolar membrane vacuole copper ion binding triose-phosphate isomerase activity	6.8e-102	AT3G55440
MDP0000322220	Heat shock cognate 70 kDa protein 1	2,1	GO:0042742 GO:0050832 GO:0006094 GO:0006096 GO:0010187 GO:0006457 GO:0046686 GO:0009409 GO:0009408 GO:0009651 GO:0009615 GO:0090332 GO:0048046 GO:0005618 GO:0009507 GO:0005737	defense response to bacterium defense response to fungus gluconeogenesis glycolysis negative regulation of seed germination protein folding response to cadmium ion response to cold response to heat response to salt stress response to virus stomatal closure apoplast cell wall chloroplast cytoplasm	1.7e-298	HSC70-1 heat shock cognate protein 70-1

			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0002020	protease binding		
			GO:0005515	protein binding		
MDP0000202749	THO complex subunit 4	2,1	GO:0008150	biological_process	4.2e-77	AT1G66080
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0003674	molecular_function		
MDP0000285549	Uncharacterized protein At4g08330, chloroplastic	2,0	GO:0008150	biological_process	6.0e-48	AT2G17705
			GO:0009507	chloroplast		
			GO:0005886	plasma membrane		
			GO:0003674	molecular_function		
MDP0000614851	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	2,0	GO:0009658	chloroplast organization	1.4e-238	CPN60A chaperonin-60alpha
			GO:0009790	embryo development		
			GO:0009793	embryo development ending in seed dormancy		
			GO:0016226	iron-sulfur cluster assembly		
			GO:0048481	ovule development		
			GO:0006457	protein folding		
			GO:0048046	apoplast		
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		
			GO:0016020	membrane		
			GO:0005739	mitochondrion		
			GO:0009579	thylakoid		
MDP0000181929	Heat shock cognate protein 80	2,0	GO:0071277	cellular response to calcium ion	8.5e-300	AT5G56010
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005886	plasma membrane		
			GO:0005524	ATP binding		
MDP0000177640	E3 ubiquitin-protein ligase RING1-like	2,0	GO:0005634	nucleus	3.0e-28	AT1G26800
			GO:0008270	zinc ion binding		
MDP0000220754	unknown protein	2,0	GO:0008150	biological_process	1.4e-128	AT4G36440
			GO:0003674	molecular_function		
MDP0000842179	Granule-bound starch synthase 2, chloroplastic/amyloplastic	2,0	GO:0010021	amylopectin biosynthetic process	4.7e-150	SS2 starch synthase 2
			GO:0019375	galactolipid biosynthetic process		
			GO:0010264	myo-inositol hexakisphosphate biosynthetic process		

			GO:0001666	response to hypoxia		
			GO:0009507	chloroplast		
			GO:0009011	starch synthase activity		
			GO:0016757	transferase activity, transferring glycosyl groups		
MDP0000290618	Peroxisome biogenesis factor 10	2,0	GO:0010381	attachment of peroxisome to chloroplast	2.3e-111	PEX10 peroxin 10
			GO:0009793	embryo development ending in seed dormancy		
			GO:0006635	fatty acid beta-oxidation		
			GO:0007031	peroxisome organization		
			GO:0009853	photorespiration		
			GO:0016558	protein import into peroxisome matrix		
			GO:0006513	protein monoubiquitination		
			GO:0005829	cytosol		
			GO:0005783	endoplasmic reticulum		
			GO:0005634	nucleus		
			GO:0005777	peroxisome		
			GO:0005515	protein binding		
			GO:0004842	ubiquitin-protein ligase activity		
			GO:0008270	zinc ion binding		
MDP0000145643	REF/SRPP-like protein At1g67360	2,0	GO:0008150	biological_process	8.4e-54	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000464933	Glutamate synthase 1 [NADH]	2,0	GO:0019676	ammonia assimilation cycle	5.4e-12	GLT1 NADH-dependent glutamate synthase 1
			GO:0048589	developmental growth		
			GO:0006094	gluconeogenesis		
			GO:0006537	glutamate biosynthetic process		
			GO:0006096	glycolysis		
			GO:0042128	nitrate assimilation		
			GO:0046686	response to cadmium ion		
			GO:0009651	response to salt stress		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005829	cytosol		
			GO:0005739	mitochondrion		
			GO:0009536	plastid		
			GO:0016040	glutamate synthase (NADH) activity		
MDP0000175599		2,0	GO:0008150	biological process	2.4e-07	AT2G42310
			GO:0009853	photorespiration		
			GO:0080129	proteasome core complex assembly		
			GO:0051788	response to misfolded protein		
			GO:0006511	ubiquitin-dependent protein catabolic process		
			GO:0009507	chloroplast		
			GO:0005747	mitochondrial respiratory chain complex I		
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000316093	Whole genome shotgun sequence of line PN40024, scaffold_71.assembly12x	2,0	GO:0006508	proteolysis	7.3e-05	AT1G50380
			GO:0010048	vernalization response		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0008236	serine-type peptidase activity		
MDP0000256438	Small heat shock protein, chloroplastic	2,0	GO:0010286	heat acclimation	1.4e-60	AT4G27670
			GO:0006457	protein folding		
			GO:0009408	response to heat		

			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009507	chloroplast		
MDP0000168932	Cysteine proteinase RD21a	2,0	GO:0005783	endoplasmic reticulum	4.1e-115	XBCP3 xylem bark cysteine peptidase 3
			GO:0005576	extracellular region		
			GO:0005773	vacuole		
			GO:0008234	cysteine-type peptidase activity		
MDP0000252405	Alpha-glucan water dikinase, chloroplastic	2,0	GO:0007623	circadian rhythm	0	SEX1 STARCH EXCESS 1
			GO:0009631	cold acclimation		
			GO:0009610	response to symbiotic fungus		
			GO:0019252	starch biosynthetic process		
			GO:0005983	starch catabolic process		
			GO:0005982	starch metabolic process		
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005739	mitochondrion		
			GO:0050521	alpha-glucan, water dikinase activity		
			GO:0005515	protein binding		
MDP0000284426	Protein transport protein SEC23	2,0	GO:0006810	transport	1.1e-263	AT5G43670
			GO:0005737	cytoplasm		
			GO:0005215	transporter activity		
MDP0000875479	unknown protein	1,9	GO:0008150	biological process	1.9e-26	AT2G27260
			GO:0005634	nucleus		
			GO:0009506	plasmodesma		
			GO:0003674	molecular function		
MDP0000284154		1,9	GO:0005634	nucleus	6.7e-10	AT3G02290
			GO:0008270	zinc ion binding		
MDP0000141863	70 kDa peptidyl-prolyl isomerase	1,9	GO:0070370	cellular heat acclimation	3.3e-224	AT3G25230
			GO:0006499	N-terminal protein myristoylation		
			GO:0006499	N-terminal protein myristoylation		
			GO:0006457	protein folding		
			GO:0000413	protein peptidyl-prolyl isomerization		
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006970	response to osmotic stress		
			GO:0009611	response to wounding		
			GO:0009845	seed germination		
			GO:0071944	cell periphery		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0016020	membrane		
			GO:0005634	nucleus		
			GO:0005516	calmodulin binding		
			GO:0005528	FK506 binding		
			GO:0003755	peptidyl-prolyl cis-trans isomerase activity		
			GO:0080025	phosphatidylinositol-3,5-bisphosphate binding		
			GO:0032266	phosphatidylinositol-3-phosphate binding		
			GO:0005515	protein binding		
MDP0000239645	C2 domain-containing protein	1,9	GO:0008150	biological process	6.0e-41	AT5G23950
			GO:0005575	cellular component		
			GO:0003674	molecular function		

MDP0000226817	EGY3 (ETHYLENE-DEPENDENT GRAVITROPISM-DEFICIENT AND YELLOW-GREEN-LIKE 3)	1,9	GO:0006457 GO:0009408 GO:0009644 GO:0042542 GO:0005739	protein folding response to heat response to high light intensity response to hydrogen peroxide mitochondrion	3.8e-206	EGY3
MDP0000161691	Heat shock protein STI	1,9	GO:0009165 GO:0046686 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0005634 GO:0005886	nucleotide biosynthetic process response to cadmium ion response to high light intensity response to hydrogen peroxide cytoplasm cytosol nucleus plasma membrane	2.1e-213	Hop2
MDP0000237464	Polyubiquitin 10	1,9	GO:0007568 GO:0006464 GO:0009751	aging cellular protein modification process response to salicylic acid	1.8e-199	AT4G05320
MDP0000209143	Uncharacterized protein R707	1,9	GO:0016051 GO:0006979 GO:0005634 GO:0016757 GO:0016758	carbohydrate biosynthetic process response to oxidative stress nucleus transferase activity, transferring glycosyl groups transferase activity, transferring hexosyl groups	1.2e-131	GolS4 galactinol synthase 4
MDP0000946614	Probable WRKY transcription factor 11	1,9	GO:0042742 GO:0035556 GO:0006355 GO:0002679 GO:0010200 GO:0005634 GO:0005516 GO:0003700	defense response to bacterium intracellular signal transduction regulation of transcription, DNA-templated respiratory burst involved in defense response response to chitin nucleus calmodulin binding sequence-specific DNA binding transcription factor activity	2.6e-76	AT4G31550
MDP0000150162	20 kDa chaperonin, chloroplastic	1,9	GO:0009658 GO:0019344 GO:0006094 GO:0006096 GO:0019288 GO:0048481 GO:1901671 GO:0046686 GO:0009409 GO:0009651 GO:0048046 GO:0009507 GO:0009941 GO:0009570 GO:0009535 GO:0005829 GO:0005739 GO:0009536 GO:0009579 GO:0005516 GO:0005507 GO:0005515	chloroplast organization cysteine biosynthetic process gluconeogenesis glycolysis isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway ovule development positive regulation of superoxide dismutase activity response to cadmium ion response to cold response to salt stress apoplast chloroplast chloroplast envelope chloroplast stroma chloroplast thylakoid membrane cytosol mitochondrion plastid thylakoid calmodulin binding copper ion binding protein binding	1,7E-86	CPN20 chaperonin 20

MDP0000879589	(+)-neomenthol dehydrogenase	1,9	GO:0042335 GO:0009409 GO:0000038 GO:0005737 GO:0009505 GO:0016491	cuticle development response to cold very long-chain fatty acid metabolic process cytoplasm plant-type cell wall oxidoreductase activity	2.4e-55	AT1G01800
MDP0000168167	Chaperone protein ClpB 2	1,9	GO:0009658 GO:0009408 GO:0009507 GO:0009570 GO:0005737 GO:0009532 GO:0005524 GO:0016887	chloroplast organization response to heat chloroplast chloroplast stroma cytoplasm plastid stroma ATP binding ATPase activity	2.9e-293	AT5G15450
MDP0000878181	unknown protein	1,9	GO:0008150 GO:0005575 GO:0003674	biological_process cellular_component molecular_function	3.3e-09	AT1G32928
MDP0000275571	Digalactosyldiacylglycerol synthase 1, chloroplastic	1,8	GO:0016117 GO:0016036 GO:0015995 GO:0019375 GO:0019288 GO:0006869 GO:0006655 GO:0042550 GO:0001666 GO:0006636 GO:0009507 GO:0009707 GO:0005739 GO:0046480 GO:0016757 GO:0035250	carotenoid biosynthetic process cellular response to phosphate starvation chlorophyll biosynthetic process galactolipid biosynthetic process isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway lipid transport phosphatidylglycerol biosynthetic process photosystem I stabilization response to hypoxia unsaturated fatty acid biosynthetic process chloroplast chloroplast outer membrane mitochondrion galactolipid galactosyltransferase activity transferase activity, transferring glycosyl groups UDP-galactosyltransferase activity	2.0e-292	DGD1 DIGALACTOSYL DIACYLGLYCEROL DEFICIENT 1
MDP0000322220	Heat shock cognate 70 kDa protein 1	1,8	GO:0042742 GO:0050832 GO:0006094 GO:0006096 GO:0010187 GO:0006457 GO:0046686 GO:0009409 GO:0009408 GO:0009651 GO:0009615 GO:0090332 GO:0048046 GO:0005618 GO:0009507 GO:0005737 GO:0005829 GO:0022626 GO:0005794	defense response to bacterium defense response to fungus gluconeogenesis glycolysis negative regulation of seed germination protein folding response to cadmium ion response to cold response to heat response to salt stress response to virus stomatal closure apoplast cell wall chloroplast cytoplasm cytosol cytosolic ribosome Golgi apparatus	1.7e-298	HSC70-1 heat shock cognate protein 70-1

			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0002020	protease binding		
			GO:0005515	protein binding		
MDP0000667723	Acylphosphatase	1,8	GO:0009507	chloroplast	3.7e-32	AT5G03370
			GO:0003998	acylphosphatase activity		
MDP0000575835	Uncharacterized calcium-binding protein At1g02270	1,8	GO:0009409	response to cold	7.3e-36	AT1G02270
			GO:0005634	nucleus		
			GO:0005509	calcium ion binding		
			GO:0003824	catalytic activity		
MDP0000440811	Cyclin-D3-2	1,8	GO:0008283	cell proliferation	1.2e-19	AT3G50070
			GO:0042023	DNA endoreduplication		
			GO:0000278	mitotic cell cycle		
			GO:0045736	negative regulation of cyclin-dependent protein serine/threonine kinase activity		
			GO:0051726	regulation of cell cycle		
			GO:0042127	regulation of cell proliferation		
			GO:0048316	seed development		
			GO:0010440	stomatal lineage progression		
			GO:0005634	nucleus		
			GO:0004693	cyclin-dependent protein serine/threonine kinase activity		
			GO:0005515	protein binding		
MDP0000264662	Chaperone protein ClpB 2	1,8	GO:0009658	chloroplast organization	0.	AT5G15450
			GO:0009408	response to heat		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0009532	plastid stroma		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
MDP0000230007	97 kDa heat shock protein	1,8	GO:0005618	cell wall	3.5e-242	Hsp70-15
			GO:0005829	cytosol		heat shock protein 70-15
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
MDP0000921426	integral membrane transporter family protein	1,8	GO:0006810	transport	5.7e-100	AT1G04570
			GO:0016020	membrane		
			GO:0005215	transporter activity		
MDP0000119199	Heat stress transcription factor A-6b	1,8	GO:0009407	toxin catabolic process	2.1e-82	AT3G22830
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000298185	Pentatricopeptide repeat-containing protein	1,8	GO:0008150	Biological process	1.8e-199	AT1G79080
			GO:0005739	mitochondrion		
MDP0000297057	Golgin candidate 6	1,7	GO:0048193	Golgi vesicle transport	1.2e-279	GC6 golgin candidate 6
			GO:0000956	nuclear-transcribed mRNA catabolic process		
			GO:0009791	post-embryonic development		
			GO:0032527	protein exit from endoplasmic reticulum		
			GO:0006486	protein glycosylation		
			GO:0009639	response to red or far red light		

			GO:0007033	vacuole organization		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005795	Golgi stack		
			GO:0005634	nucleus		
MDP0000192625	Putative serine/threonine-protein kinase receptor	1,7	GO:0005886	plasma membrane	1.6e-150	AT2G19130
			GO:0004672	protein kinase activity		
MDP0000536661		1,7	GO:0034968	histone lysine methylation	1.5e-07	PRMT10 protein arginine methyltransferase 10
			GO:0019919	peptidyl-arginine methylation, to asymmetrical-dimethyl arginine		
			GO:0009909	regulation of flower development		
			GO:0010228	vegetative to reproductive phase transition of meristem		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
			GO:0008469	histone-arginine N-methyltransferase activity		
			GO:0008168	methyltransferase activity		
			GO:0035242	protein-arginine omega-N asymmetric methyltransferase activity		
			GO:0035241	protein-arginine omega-N monomethyltransferase activity		
			GO:0016277	[myelin basic protein]-arginine N-methyltransferase activity		
MDP0000136296	BI1-like protein	1,7	GO:0008150	biological_process	9.8e-94	AT1G03070
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0016595	glutamate binding		
MDP0000176947	Probable LRR receptor-like serine/threonine-protein kinase	1,7	GO:0046685	response to arsenic-containing substance	1.3e-14	AT2G30500
MDP0000199234	Uncharacterized abhydrolase domain-containing protein DDB_G0269086	1,7	GO:0042538	hyperosmotic salinity response	3.1e-123	AT1G73480
			GO:0009737	response to abscisic acid		
			GO:0009409	response to cold		
			GO:0009414	response to water deprivation		
			GO:0005737	cytoplasm		
			GO:0016787	hydrolase activity		
MDP0000833759	DnaJ protein homolog	1,7	GO:0006499	N-terminal protein myristoylation	1.8e-160	J3
			GO:0048573	photoperiodism, flowering		
			GO:0009911	positive regulation of flower development		
			GO:0006457	protein folding		
			GO:0043462	regulation of ATPase activity		
			GO:0009651	response to salt stress		
			GO:0005618	cell wall		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005730	nucleolus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
MDP0000208958	15.7 kDa heat shock protein, peroxisomal	1,7	GO:0006457	protein folding	7.0e-43	AT5G37670
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0000302	response to reactive oxygen species		

			GO:0005737	cytoplasm		
			GO:0005782	peroxisomal matrix		
			GO:0003674	molecular_function		
MDP0000256746	DnaJ homolog subfamily C member 3 homolog	1,7	GO:0006457	protein folding	1.2e-151	AT5G03160
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005783	endoplasmic reticulum		
			GO:0005788	endoplasmic reticulum lumen		
			GO:0005886	plasma membrane		
MDP0000807190	ABC transporter B family member 26, chloroplastic	1,7	GO:0015996	chlorophyll catabolic process	5.4e-267	AT1G70610
			GO:0055072	iron ion homeostasis		
			GO:0010039	response to iron ion		
			GO:0000302	response to reactive oxygen species		
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0042626	ATPase activity, coupled to transmembrane movement of substances		
			GO:0005215	transporter activity		
MDP0000158047	EXO (EXORDIUM)	1,7	GO:0009741	response to brassinosteroid	8.2e-102	AT4G08950
			GO:0016126	sterol biosynthetic process		
			GO:0005618	cell wall		
			GO:0005829	cytosol		
			GO:0005576	extracellular region		
			GO:0005794	Golgi apparatus		
			GO:0009505	plant-type cell wall		
			GO:0003674	Molecularfunction		
MDP0000304623	Chaperone protein ClpB 2	1,7	GO:0006457	protein folding	0	AT2G25140
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005739	mitochondrion		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
MDP0000232642	Protein translation factor SUI1 homolog	1,7	GO:0006413	translational initiation	8.2e-46	AT5G54940
			GO:0003743	translation initiation factor activity		
MDP0000264662	Chaperone protein ClpB 2	1,7	GO:0009658	chloroplast organization	0.	AT5G15450
			GO:0009408	response to heat		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0009532	plastid stroma		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
MDP0000157315	Programmed cell death protein 4	1,7	GO:0008150	biological_process	2.0e-202	AT1G22730
			GO:0005634	nucleus		
			GO:0003674	molecular_function		
MDP0000322220	Heat shock cognate 70 kDa protein 1	1,7	GO:0042742	defense response to bacterium	1.7e-298	HSC70-1 heat shock cognate protein 70-1
			GO:0050832	defense response to fungus		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0010187	negative regulation of seed germination		

			GO:0006457	protein folding		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009651	response to salt stress		
			GO:0009615	response to virus		
			GO:0090332	stomatal closure		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0002020	protease binding		
			GO:0005515	protein binding		
MDP0000362505	17.9 kDa class II heat shock protein	1,7	GO:0006457	protein folding	1.3e-33	HSP17.6II
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000455474	Ubiquitin-60S ribosomal protein L40	1,7	GO:0001510	RNA methylation	2.7e-63	UBQ2 ubiquitin extension protein 2
			GO:0022625	cytosolic large ribosomal subunit		
			GO:0005730	nucleolus		

Downregulated genes						
MDP0000238081	Allene oxide cyclase 4, chloroplastic	0,5	GO:0009695	jasmonic acid biosynthetic process	3.4e-61	AOC4 allene oxide cyclase 4
			GO:0009507	chloroplast		
			GO:0005886	plasma membrane		
			GO:0046423	allene-oxide cyclase activity		
MDP0000305472	Pentatricopeptide repeat-containing protein At1g28690, mitochondrial	0,5	GO:0008150	biological process	2.3e-117	AT1G28690
			GO:0005739	mitochondrion		
MDP0000286457	Probable serine/threonine-protein kinase Cx32, chloroplastic	0,5	GO:0006995	cellular response to nitrogen starvation	5.6e-125	Kin3 kinase 3
			GO:0050832	defense response to fungus		
			GO:0045087	innate immune response		
			GO:0031348	negative regulation of defense response		
			GO:0031347	regulation of defense response		
			GO:0010200	response to chitin		
			GO:0009697	salicylic acid biosynthetic process		
			GO:0009863	salicylic acid mediated signaling pathway		
			GO:0009627	systemic acquired resistance		
			GO:0009507	chloroplast		
			GO:0005886	plasma membrane		
			GO:0016301	kinase activity		
MDP0000266638	Squalene monooxygenase	0,5	GO:0016132	brassinosteroid biosynthetic process	4.2e-171	XF1

			GO:0019745	pentacyclic triterpenoid biosynthetic process		
			GO:0000271	polysaccharide biosynthetic process		
			GO:0009414	response to water deprivation		
			GO:0016126	sterol biosynthetic process		
			GO:0005576	extracellular region		
			GO:0004506	squalene monooxygenase activity		
MDP0000939633	AP2/ERF and B3 domain-containing transcription repressor TEM1	0,5	GO:0030003	cellular cation homeostasis	3.7e-95	TEM1 TEMPRANILLO 1
			GO:0070838	divalent metal ion transport		
			GO:0009873	ethylene-activated signaling pathway		
			GO:0048573	photoperiodism, flowering		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000425402	Transmembrane protein 131	0,5	GO:0008150	biological process	8.2e-36	AT5G66820
			GO:0009086	methionine biosynthetic process		
			GO:0000394	RNA splicing, via endonucleolytic cleavage and ligation		
			GO:0010048	vernalization response		
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000273394	1-aminocyclopropane-1-carboxylate oxidase homolog 4	0,5	GO:0005737	cytoplasm	1.2e-102	AT1G06650
			GO:0005829	cytosol		
			GO:0016706	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors		
MDP0000187921	Protein TIFY 10A	0,5	GO:0009738	abscisic acid-activated signaling pathway	5.4e-45	JAZ1 jasmonate-zim-domain protein 1
			GO:0007154	cell communication		
			GO:0042742	defense response to bacterium		
			GO:0050832	defense response to fungus		
			GO:0030968	endoplasmic reticulum unfolded protein response		
			GO:0009908	flower development		
			GO:0042538	hyperosmotic salinity response		
			GO:0035556	intracellular signal transduction		
			GO:0009695	jasmonic acid biosynthetic process		
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0000165	MAPK cascade		
			GO:0031348	negative regulation of defense response		
			GO:0043069	negative regulation of programmed cell death		
			GO:0009555	pollen development		
			GO:0006612	protein targeting to membrane		
			GO:0010363	regulation of plant-type hypersensitive response		
			GO:0009733	response to auxin		
			GO:0010200	response to chitin		
			GO:0009409	response to cold		
			GO:0009723	response to ethylene		
			GO:0009620	response to fungus		
			GO:0009753	response to jasmonic acid		
			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0009863	salicylic acid mediated signaling pathway		

			GO:0007165	signal transduction		
			GO:0009862	systemic acquired resistance, salicylic acid mediated signaling pathway		
			GO:0005634	nucleus		
			GO:0005515	protein binding		
MDP0000312316	IAA-amino acid hydrolase ILR1-like 4	0,5	GO:0009793	embryo development ending in seed dormancy	7.2e-193	emb2004 embryo defective 2004
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0005739	mitochondrion		
			GO:0009536	plastid		
MDP0000135939	N/A	0,5	GO:0009738	abscisic acid-activated signaling pathway	0.00012	AT5G65300
			GO:0008150	biological process		
			GO:0009693	ethylene biosynthetic process		
			GO:0035556	intracellular signal transduction		
			GO:0010200	response to chitin		
			GO:0009723	response to ethylene		
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000830926	Probable gibberellin receptor GID1L3	0,5	GO:0006865	amino acid transport	7.5e-44	AT5G06570
			GO:0005634	nucleus		
MDP0000288983	Indole-3-acetic acid-induced protein ARG7	0,5	GO:0042742	defense response to bacterium	1.1e-23	AT4G38840
			GO:0009733	response to auxin		
			GO:0009409	response to cold		
			GO:0009507	chloroplast		
			GO:0005886	plasma membrane		
			GO:0003674	molecular function		
MDP0000309944	Dof zinc finger protein DOF3.7	0,5	GO:0042398	cellular modified amino acid biosynthetic process	1.2e-50	AT2G46590
			GO:0009698	phenylpropanoid metabolic process		
			GO:0006598	polyamine catabolic process		
			GO:0009409	response to cold		
			GO:0009416	response to light stimulus		
			GO:0009845	seed germination		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000565690	Protein TIFY 11B	0,5	GO:0009738	abscisic acid-activated signaling pathway	1.4e-20	JAZ5 jasmonate-zim-domain protein 5
			GO:0009693	ethylene biosynthetic process		
			GO:0042538	hyperosmotic salinity response		
			GO:0009695	jasmonic acid biosynthetic process		
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0009733	response to auxin		
			GO:0010200	response to chitin		
			GO:0009723	response to ethylene		
			GO:0009620	response to fungus		
			GO:0009753	response to jasmonic acid		
			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0007165	signal transduction		
			GO:0005634	nucleus		
			GO:0005515	protein binding		
MDP0000334850	wound-responsive protein-related	0,5	GO:0008150	biological process	5.2e-14	AT4G28240
			GO:0005634	nucleus		
			GO:0003674	molecular function		

MDP0000565690	Protein TIFY 11B	0,5	GO:0009738 GO:0009693 GO:0042538 GO:0009695 GO:0009867 GO:0009733 GO:0010200 GO:0009723 GO:0009620 GO:0009753 GO:0009414 GO:0009611 GO:0007165 GO:0005634 GO:0005515	abscisic acid-activated signaling pathway ethylene biosynthetic process hyperosmotic salinity response jasmonic acid biosynthetic process jasmonic acid mediated signaling pathway response to auxin response to chitin response to ethylene response to fungus response to jasmonic acid response to water deprivation response to wounding signal transduction nucleus protein binding	1.4e-20	JAZ5 jasmonate-zim-domain protein 5
MDP0000362286	Auxin-repressed 12.5 kDa protein	0,5	GO:0005634	nucleus	4.1e-24	DRM2 dormancy associated gene 2
MDP0000372629	unknown protein	0,5	GO:0009062 O:0009739 GO:0005634 GO:0003674	fatty acid catabolic process response to gibberellin nucleus molecular function	1.3e-21	AT2G34340
MDP0000649644	unknown protein	0,5	GO:0008150 GO:0005739 GO:0003674	biological process mitochondrion molecular function	3.3e-18	AT1G58420
MDP0000047589	Beta-glucosidase 12	0,5	GO:0010359 GO:0005618 GO:0005829 GO:0005576 GO:0005794 GO:0009505 GO:0009506 GO:0004553	regulation of anion channel activity cell wall cytosol extracellular region Golgi apparatus plant-type cell wall plasmodesma hydrolase activity, hydrolyzing O-glycosyl compounds	1.0e-143	AT2G44450
MDP0000911607	L-galactono-1,4-lactone dehydrogenase, mitochondrial	0,5	GO:0019853 GO:0055114 GO:0005739 GO:0050105	L-ascorbic acid biosynthetic process oxidation-reduction process mitochondrion L-gulonolactone oxidase activity	7.7e-64	GulLO3 L -gulono-1,4-lactone (L -GulL) oxidase 3
MDP0000187921	Protein TIFY 10A	0,5	GO:0009738 GO:0007154 GO:0042742 GO:0050832 GO:0030968 GO:0009908 GO:0042538 GO:0035556 GO:0009695 GO:0009867 GO:0000165 GO:0031348 GO:0043069 GO:0009555	abscisic acid-activated signaling pathway cell communication defense response to bacterium defense response to fungus endoplasmic reticulum unfolded protein response flower development hyperosmotic salinity response intracellular signal transduction jasmonic acid biosynthetic process jasmonic acid mediated signaling pathway MAPK cascade negative regulation of defense response negative regulation of programmed cell death pollen development	5.4e-45	JAZ1 jasmonate-zim-domain protein 1

			GO:0006612	protein targeting to membrane		
			GO:0010363	regulation of plant-type hypersensitive response		
			GO:0009733	response to auxin		
			GO:0010200	response to chitin		
			GO:0009409	response to cold		
			GO:0009723	esponse to ethylene		
			GO:0009620	response to fungus		
			GO:0009753	response to jasmonic acid		
			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0009863	salicylic acid mediated signaling pathway		
			GO:0007165	signal transduction		
			GO:0009862	systemic acquired resistance, salicylic acid mediated signaling pathway		
			GO:0005634	nucleus		
			GO:0005515	protein binding		
MDP0000784851	unknown protein	0,5	GO:0008150	biological process	2.7e-19	AT1G49000
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000118810	Probable WRKY transcription factor 71	0,5	GO:0006865	amino acid transport	3.4e-47	AT4G18170
			GO:0006355	regulation of transcription, DNA-templated		
			GO:0010583	response to cyclopentenone		
			GO:0009407	toxin catabolic process		
			GO:0005634	nucleus		
			GO:0043565	sequence-specific DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000292277	Predicted protein	0,5	GO:0008150	biological process	1.2e-09	AT1G58420
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000187921	Protein TIFY 10A	0,4	GO:0009738	abscisic acid-activated signaling pathway	5.4e-45	JAZ1 jasmonate-zim-domain protein 1
			GO:0007154	cell communication		
			GO:0042742	defense response to bacterium		
			GO:0050832	defense response to fungus		
			GO:0030968	endoplasmic reticulum unfolded protein response		
			GO:0009908	flower development		
			GO:0042538	hyperosmotic salinity response		
			GO:0035556	intracellular signal transduction		
			GO:0009695	jasmonic acid biosynthetic process		
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0000165	MAPK cascade		
			GO:0031348	negative regulation of defense response		
			GO:0043069	negative regulation of programmed cell death		
			GO:0009555	pollen development		
			GO:0006612	protein targeting to membrane		
			GO:0010363	regulation of plant-type hypersensitive response		
			GO:0009733	response to auxin		
			GO:0010200	response to chitin		
			GO:0009409	response to cold		
			GO:0009723	response to ethylene		
			GO:0009620	response to fungus		
			GO:0009753	response to jasmonic acid		

			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0009863	salicylic acid mediated signaling pathway		
			GO:0007165	signal transduction		
			GO:0009862	systemic acquired resistance, salicylic acid mediated signaling pathway		
			GO:0005634	nucleus		
			GO:0005515	protein binding		
MDP0000120098	Mannan endo-1,4-beta-mannosidase 6	0,4	GO:0009845	seed germination	1.0e-157	AT5G01930
			GO:0005576	extracellular region		
			GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds		
MDP0000257764	Auxin-induced protein X10A	0,4	GO:0042742	defense response to bacterium	6.9e-22	AT4G38840
			GO:0009733	response to auxin		
			GO:0009409	response to cold		
			GO:0009507	chloroplast		
			GO:0005886	plasma membrane		
			GO:0003674	molecular function		
MDP0000603546	Transcription factor bHLH14	0,4	GO:0009718	anthocyanin-containing compound biosynthetic process	4.8e-66	AT4G17880
			GO:0006952	defense response		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006355	regulation of transcription, DNA-templated		
			GO:0005634	nucleus		
			GO:0043425	bHLH transcription factor binding		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000257621		0,4	GO:0030865	cortical cytoskeleton organization	5.1e-16	EB1C end binding protein 1C
			GO:0016572	histone phosphorylation		
			GO:0000278	mitotic cell cycle		
			GO:0009652	thigmotropism		
			GO:0010228	vegetative to reproductive phase transition of meristem		
			GO:0005618	cell wall		
			GO:0005737	cytoplasm		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0009524	phragmoplast		
			GO:0005819	spindle		
			GO:0008017	microtubule binding		
MDP0000565690	Protein TIFY 11B	0,4	GO:0009738	abscisic acid-activated signaling pathway	1.4e-20	JAZ5 jasmonate-zim-domain protein 5
			GO:0009693	ethylene biosynthetic process		
			GO:0042538	hyperosmotic salinity response		
			GO:0009695	jasmonic acid biosynthetic process		
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0009733	response to auxin		
			GO:0010200	response to chitin		
			GO:0009723	response to ethylene		
			GO:0009620	response to fungus		
			GO:0009753	response to jasmonic acid		
			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0007165	signal transduction		
			GO:0005634	nucleus		

			GO:0005515	protein binding		
MDP0000242554	Transcription factor ATR2	0,4	GO:0009718	anthocyanin-containing compound biosynthetic process	4.0e-57	AT5G46760
			GO:0006952	defense response		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006355	regulation of transcription, DNA-templated		
			GO:0009753	response to jasmonic acid		
			GO:0005634	nucleus		
			GO:0043425	bHLH transcription factor binding		
			GO:0003677	DNA binding		
			GO:0005515	protein binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000805271	Probable receptor-like protein kinase At1g11050	0,4	GO:0009738	abscisic acid-activated signaling pathway	1.3e-148	AT1G11050
			GO:0009693	ethylene biosynthetic process		
			GO:0035556	intracellular signal transduction		
			GO:0009723	response to ethylene		
			GO:0005886	plasma membrane		
			GO:0016301	kinase activity		
MDP0000274386	Epsin-3	0,4	GO:0008150	biological process	2.3e-35	AT3G23350
			GO:0005634	nucleus		
			GO:0003674	molecular function		

Table 6

Transcripts differentially expressed in HT fruit compare to the control after 4 hours from the treatment.

MDP	Description	Ratio	GO code	Go terms	*p value*	*Arabidopsis* protein
Upregulated genes						
MDP0000302297	Somatic embryogenesis receptor kinase 1	3,1	GO:0006355	regulation of transcription, DNA-templated	1.4e-75	B-box domain protein 12, BBX12
			GO:0005634	nucleus		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
			GO:0008270	zinc ion binding		
MDP0000291831	17.9 kDa class II heat shock protein	2,7	GO:0006457	protein folding	1.5e-36	HSP17.6II
			GO:0034976	response to endoplasmic reticulum stress		17.6 kDa class II heat shock protein
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000271554	HSA32 (HEAT-STRESS-ASSOCIATED 32); catalytic	2,6	GO:0010286	heat acclimation	7.3e-114	HSA32
			GO:0008152	metabolic process		AT4G21320
			GO:0010608	posttranscriptional regulation of gene expression		
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005618	cell wall		
			GO:0005634	nucleus		
			GO:0004252	serine-type endopeptidase activity		
MDP0000878773	1-aminocyclopropane-1-carboxylate oxidase homolog 1	2,6	GO:0042538	hyperosmotic salinity response	6.0e-80	AT1G06620
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0009414	response to water deprivation		
			GO:0009611	response to wounding		
			GO:0005737	cytoplasm		
			GO:0016706	oxidoreductase activity		
MDP0000657396	Protein FAM32A	2,6	GO:0008150	biological process	1.8e-18	AT1G16810
			GO:0005634	nucleus		
			GO:0003674	molecular unction		
MDP0000311339	Heat shock 70 kDa protein	2,5	GO:0010286	heat acclimation	2.4e-59	Hsp70b
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009615	response to virus		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0016020	membrane		
			GO:0005886	plasma membrane		
MDP0000290546	DnaJ homolog subfamily B member 13	2,5	GO:0006457	protein folding	4.1e-120	
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000129571		2,5	GO:0030154	cell differentiation	0.090	RGF6

			GO:0048527	lateral root development		AT4G16515
			GO:0009958	positive gravitropism		
			GO:0008284	positive regulation of cell proliferation		
			GO:2000012	regulation of auxin polar transport		
			GO:0032880	regulation of protein localization		
			GO:0022622	root system development		
			GO:0005576	extracellular region		
			GO:0005615	extracellular space		
			GO:0008083	growth factor activity		
			GO:0003674	molecular function		
MDP0000145643	REF/SRPP-like protein At1g67360	2,4	GO:0008150	biological process	8.4e-54	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000611170	Endoplasmin homolog	2,4	GO:0006094	gluconeogenesis	3.6e-313	SHD AT4G24190
			GO:0006096	glycolysis		
			GO:0007030	Golgi organization		
			GO:0006972	hyperosmotic response		
			GO:0006499	N-terminal protein myristoylation		
			GO:0006457	protein folding		
			GO:0009306	protein secretion		
			GO:0010075	regulation of meristem growth		
			GO:0009934	regulation of meristem structural organization		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009651	response to salt stress		
			GO:0009266	response to temperature stimulus		
			GO:0009414	response to water deprivation		
			GO:0006833	water transport		
			GO:0048046	apoplast		
			GO:0009507	chloroplast		
			GO:0005829	cytosol		
			GO:0005783	endoplasmic reticulum		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005739	mitochondrion		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0005773	vacuole		
			GO:0005524	ATP binding		
MDP0000684170	Heat shock cognate 70 kDa protein 4	2,4	GO:0010286	heat acclimation	7.2e-217	heat-shock protein 70T-2
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000121482	Probable LRR receptor-like serine/threonine-protein kinase	2,3	GO:0009718	anthocyanin-containing compound biosynthetic process	5.3e-131	AT3G03770
			GO:0006468	protein phosphorylation		
			GO:0009744	response to sucrose		
			GO:0010224	response to UV-B		
			GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway		
			GO:0005886	plasma membrane		
			GO:0005524	ATP binding		
			GO:0004672	protein kinase activity		
			GO:0004674	protein serine/threonine kinase		

				activity		
MDP0000189960	unknown	2,3	GO:0052546 GO:0052541 GO:0016126 GO:0005634 GO:0003674	cell wall pectin metabolic process plant-type cell wall cellulose metabolic process sterol biosynthetic process nucleus molecular function	1.8e-55	AT2G35880
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	2,3	GO:0016049 GO:0009793 GO:0007030 GO:0048507 GO:0009933 GO:0045053 GO:0010016 GO:0005829 GO:0005794 GO:0003674	cell growth embryo development ending in seed dormancy Golgi organization meristem development meristem structural organization protein retention in Golgi apparatus shoot system morphogenesis cytosol Golgi apparatus molecular function	2.5e-167	AT5G51430
MDP0000379307	97 kDa heat shock protein	2,3	GO:0005618 GO:0005829 GO:0005634 GO:0005886 GO:0009506	cell wall cytosol nucleus plasma membrane plasmodesma	4.9e-92	Hsp70-15
MDP0000161691	Heat shock protein STI	2,3	GO:0009165 GO:0046686 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0005634 GO:0005886	nucleotide biosynthetic process response to cadmium ion response to high light intensity response to hydrogen peroxide cytoplasm cytosol nucleus plasma membrane	2.1e-213	Hop2
MDP0000122734	Heat shock cognate 70 kDa protein 4	2,3	GO:0006457 GO:0016567 GO:0009617 GO:0046686 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0009266 GO:0009615 GO:0048046 GO:0005618 GO:0005829 GO:0005794 GO:0005739 GO:0005886 GO:0005774 GO:0005524 GO:0031625	protein folding protein ubiquitination response to bacterium response to cadmium ion response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide response to temperature stimulus response to virus apoplast cell wall cytosol Golgi apparatus mitochondrion plasma membrane vacuolar membrane ATP binding ubiquitin protein ligase binding	1.8e-256	AT3G12580
MDP0000239645	C2 domain-containing protein	2,3	GO:0008150 GO:0005575 GO:0003674	biological process cellular component molecular function	6.0e-41	AT5G23950
MDP0000720039	Polyubiquitin	2,3	GO:0006464 GO:0042787 GO:0006511	cellular protein modification process protein ubiquitination involved in ubiquitin-dependent protein catabolic process ubiquitin-dependent protein catabolic process	1.4e-135	AT5G20620
MDP0000207407	18.5 kDa class I heat shock protein	2,3	GO:0010286 GO:0006457	heat acclimation protein folding	5.3e-42	AT2G29500

			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000755113	Probable glutathione S-transferase	2,2	GO:0016036	cellular response to phosphate starvation	1.4e-85	AT1G78380
			GO:0042631	cellular response to water deprivation		
			GO:0006635	fatty acid beta-oxidation		
			GO:0019375	galactolipid biosynthetic process		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0080129	proteasome core complex assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0046686	response to cadmium ion		
			GO:0010583	response to cyclopentenone		
			GO:0051788	response to misfolded protein		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0043295	glutathione binding		
			GO:0004364	glutathione transferase activity		
MDP0000137929	Ammonium transporter 3 member 1	2,2	GO:0009738	abscisic acid-activated signaling pathway	4.6e-190	AMT2 ammonium transporter 2
			GO:0043090	amino acid import		
			GO:0015696	ammonium transport		
			GO:0006820	anion transport		
			GO:0015802	basic amino acid transport		
			GO:0007154	cell communication		
			GO:0006995	cellular response to nitrogen starvation		
			GO:0042742	defense response to bacterium		
			GO:0050832	defense response to fungus		
			GO:0009595	detection of biotic stimulus		
			GO:0009581	detection of external stimulus		
			GO:0030968	endoplasmic reticulum unfolded protein response		
			GO:0006888	ER to Golgi vesicle-mediated transport		
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0000165	MAPK cascade		
			GO:0031348	negative regulation of defense response		
			GO:0043069	negative regulation of programmed cell death		
			GO:0006862	nucleotide transport		
			GO:0015695	organic cation transport		
			GO:0006612	protein targeting to membrane		
			GO:0031347	regulation of defense response		
			GO:0010310	regulation of hydrogen peroxide metabolic process		
			GO:0043269	regulation of ion transport		

			GO:0043900	regulation of multi-organism process		
			GO:0010363	regulation of plant-type hypersensitive response		
			GO:0010200	response to chitin		
			GO:0009409	response to cold		
			GO:0009750	response to fructose		
			GO:0009749	response to glucose		
			GO:0002237	response to molecule of bacterial origin		
			GO:0009624	response to nematode		
			GO:0051707	response to other organism		
			GO:0009744	response to sucrose		
			GO:0009697	salicylic acid biosynthetic process		
			GO:0009863	salicylic acid mediated signaling pathway		
			GO:0009627	systemic acquired resistance (SAR)		
			GO:0009862	SAR, salicylic acid mediated signaling pathway		
			GO:0005737	cytoplasm		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0008519	ammonium transmembrane transporter activity		
			GO:0015398	high affinity secondary active ammonium transmembrane transporter activity		
MDP0000275263	THO complex subunit 4	2,2	GO:0008150	biological process	2.6e-61	AT1G66080
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0003674	molecular function		
MDP0000436577	Rac-like GTP-binding protein ARAC1	2,2	GO:0009738	abscisic acid-activated signaling pathway	2.3e-94	ARAC1 AT2G17800
			GO:0007015	actin filament organization		
			GO:0009734	auxin-activated signaling pathway		
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0009524	phragmoplast		
			GO:0005886	plasma membrane		
			GO:0005819	spindle		
			GO:0005525	GTP binding		
MDP0000800581	glycine-rich protein	2,2	GO:0005886	plasma membrane	1.4e-40	AT4G22740
			GO:0003674	molecular function		
MDP0000160372	REF/SRPP-like protein At1g67360	2,2	GO:0008150	biological process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000314161	Cyclin-A2-3	2,2	GO:0008283	cell proliferation	4.9e-26	CYCLIN A2;3
			GO:0000911	cytokinesis by cell plate formation		
			GO:0042023	DNA endoreduplication		
			GO:0010311	lateral root formation		
			GO:0000226	microtubule cytoskeleton organization		
			GO:0000278	mitotic cell cycle		
			GO:0045736	negative regulation of cyclin-dependent protein serine/threonine kinase activity		
			GO:0000280	nuclear division		
			GO:2000123	positive regulation of stomatal complex development		

			GO:0043248	proteasome assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0051726	regulation of cell cycle		
			GO:0006275	regulation of cell cycle		
			GO:0010389	regulation of G2/M transition of mitotic cell cycle		
			GO:0051510	regulation of unidimensional cell growth		
			GO:0051788	response to misfolded protein		
			GO:0051225	spindle assembly		
			GO:0010440	stomatal lineage progression		
			GO:0005634	nucleus		
			GO:0016538	cyclin-dependent protein serine/threonine kinase regulator activity		
			GO:0005515	protein binding		
MDP0000574524	17.5 kDa class I heat shock protein	2,2	GO:0010286	heat acclimation	6.1e-28	AT2G29500
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000203499	Endoplasmin homolog	2,2	GO:0006094	gluconeogenesis	3.5e-266	SHD AT4G24190
			GO:0006096	glycolysis		
			GO:0007030	Golgi organization		
			GO:0006972	hyperosmotic response		
			GO:0006499	N-terminal protein myristoylation		
			GO:0006457	protein folding		
			GO:0009306	protein secretion		
			GO:0010075	regulation of meristem growth		
			GO:0009934	regulation of meristem structural organization		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009651	response to salt stress		
			GO:0009266	response to temperature stimulus		
			GO:0009414	response to water deprivation		
			GO:0006833	water transport		
			GO:0048046	apoplast		
			GO:0009507	chloroplast		
			GO:0005829	cytosol		
			GO:0005783	endoplasmic reticulum		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005739	mitochondrion		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0005773	vacuole		
			GO:0005524	ATP binding		
MDP0000209621	unknown protein	2,1	GO:0008150	biological_process	7.7e-41	AT5G47830
			GO:0006457	protein folding		

			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
			GO:0005634	nucleus		
			GO:0003674	molecular_function		
MDP0000768256	Hsp70-binding protein 1	2,1	GO:0006457	protein folding	2.7e-132	Fes1A
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009651	response to salt stress		
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0030544	Hsp70 protein binding		
MDP0000267891	UPF0098 protein MTH_273	2,1	GO:0008150	biological_process	4.4e-54	AT5G01300
			GO:0010264	myo-inositol hexakisphosphate biosynthetic process		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0010162	seed dormancy process		
			GO:0005737	cytoplasm		
			GO:0008429	phosphatidylethanolamine binding		
MDP0000141863	70 kDa peptidyl-prolyl isomerase	2,1	GO:0070370	cellular heat acclimation	3.3e-224	ROF1 AT3G25230
			GO:0006499	N-terminal protein myristoylation		
			GO:0006499	N-terminal protein myristoylation		
			GO:0006457	protein folding		
			GO:0000413	protein peptidyl-prolyl isomerization		
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006970	response to osmotic stress		
			GO:0009611	response to wounding		
			GO:0009845	seed germination		
			GO:0071944	cell periphery		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0016020	membrane		
			GO:0005634	nucleus		
			GO:0005516	calmodulin binding		
			GO:0005528	FK506 binding		
			GO:0003755	peptidyl-prolyl cis-trans isomerase activity		
			GO:0080025	phosphatidylinositol-3,5-bisphosphate binding		
			GO:0032266	phosphatidylinositol-3-phosphate binding		
			GO:0005515	protein binding		
MDP0000303430	Heat shock protein 83	2,1	GO:0006457	protein folding	1.6e-275	Hsp81.4 AT5G56000
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
MDP0000767855	Delta-1-pyrroline-5-carboxylate	2,1	GO:0010133	proline catabolic process to glutamate	7.8e-51	ALDH12A1 AT5G62530

	dehydrogenase 12A1, mitochondrial		GO:0006560 GO:0072593 GO:0009651 GO:0009507 GO:0005739 GO:0003842 GO:0004028 GO:0050897 GO:0008270	proline metabolic process reactive oxygen species metabolic process response to salt stress chloroplast mitochondrion 1-pyrroline-5-carboxylate dehydrogenase activity 3-chloroallyl aldehyde dehydrogenase activity cobalt ion binding zinc ion binding		
MDP0000796348	Scarecrow-like protein 33	2,1	GO:0000956 GO:0045893 GO:0006486 GO:0006355 GO:0009410 GO:0005829 GO:0005634 GO:0003700	nuclear-transcribed mRNA catabolic process positive regulation of transcription, DNA-templated protein glycosylation regulation of transcription, DNA-templated response to xenobiotic stimulus cytosol nucleus sequence-specific DNA binding transcription factor activity	2.1e-147	SCL14 SCARECROW-like 14
MDP0000508644	unknown protein	2,1	GO:0008150 GO:0030968 GO:0010286 GO:0043069 GO:0006612 GO:0010363 GO:0005576 GO:0005739	biological_process endoplasmic reticulum unfolded protein response heat acclimation negative regulation of programmed cell death protein targeting to membrane regulation of plant-type hypersensitive response extracellular region mitochondrion	1.8e-20	AT5G10695
MDP0000446914	Glycogenin-1	2,1	GO:0016051 GO:0006012 GO:0009737 GO:0009409 GO:0009408 GO:0009644 GO:0042542 GO:0006979 GO:0009651 GO:0009414 GO:0005575 GO:0047216 GO:0016758	carbohydrate biosynthetic process galactose metabolic process response to abscisic acid response to cold response to heat response to high light intensity response to hydrogen peroxide response to oxidative stress response to salt stress response to water deprivation cellular component inositol 3-alpha-galactosyltransferase activity transferase activity, transferring hexosyl groups	1.1e-148	GolS1 AT2G47180
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	2,1	GO:0016049 GO:0009793 GO:0007030 GO:0048507 GO:0009933 GO:0045053 GO:0010016 GO:0005829 GO:0005794 GO:0003674	cell growth embryo development ending in seed dormancy Golgi organization meristem development meristem structural organization protein retention in Golgi apparatus shoot system morphogenesis cytosol Golgi apparatus molecular function	2.5e-167	EYE
MDP0000549793	ATERDJ3A; oxidoreductase	2,1	GO:0009688 GO:0034605 GO:0010286 GO:0009860	abscisic acid biosynthetic process cellular response to heat heat acclimation pollen tube growth	4.9e-131	ATERDJ3A

			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005788	endoplasmic reticulum lumen		
			GO:0009506	plasmodesma		
			GO:0016491	oxidoreductase activity		
MDP0000186670		2,1	GO:0006457	protein folding	1.5e-10	AT1G53540
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000154937	Sn1-specific diacylglycerol lipase beta	2,1	GO:0006629	lipid metabolic process	3.4e-166	AT2G42450
			GO:0005886	plasma membrane		
			GO:0004806	triglyceride lipase activity		
MDP0000466683	Glycogenin-1	2,0	GO:0016051	carbohydrate biosynthetic process	1.5e-148	GolS1
			GO:0006012	galactose metabolic process		AT2G47180
			GO:0009737	response to abscisic acid		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxid		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009414	response to water deprivation		
			GO:0005575	cellular component		
			GO:0047216	inositol 3-alpha-galactosyltransferase activity		
			GO:0016758	transferase activity, transferring hexosyl groups		
MDP0000700383	17.9 kDa class II heat shock protein	2,0	GO:0006457	protein folding	8.8e-35	HSP17.6II
			GO:0034976	response to endoplasmic reticulum stress		17.6 kDa class II heat shock protein
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000707429	transferase, transferring glycosyl groups	2,0	GO:0005576	extracellular region	6.0e-105	AT3G18180
MDP0000171695	unknown protein	2,0	GO:0009061	anaerobic respiration	1.9e-30	AT3G10020
			GO:0006979	response to oxidative stress		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000160372	REF/SRPP-like protein At1g67360	2,0	GO:0008150	biological process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000543224	Probable pyridoxal biosynthesis protein PDX1.2	2,0	GO:0006457	protein folding	1.1e-111	PDX1.2, pyridoxine biosynthesis 1.2
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0042819	vitamin B6 biosynthetic process		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005515	protein binding		
			GO:0046982	protein heterodimerization activity		
MDP0000122734	Heat shock cognate 70 kDa protein 4	2,0	GO:0006457	protein folding	1.8e-256	HSP70
			GO:0016567	protein ubiquitination		AT3G12580
			GO:0009617	response to bacterium		

			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009266	response to temperature stimulus		
			GO:0009615	response to virus		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005739	mitochondrion		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
			GO:0031625	ubiquitin protein ligase binding		
MDP0000152242	Triosephosphate isomerase, cytosolic	2,0	GO:0009060	aerobic respiration	6.8e-102	TPI AT3G55440
			GO:0044262	cellular carbohydrate metabolic process		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0019344	cysteine biosynthetic process		
			GO:0007030	Golgi organization		
			GO:0006972	hyperosmotic response		
			GO:0008152	metabolic process		
			GO:0006098	pentose-phosphate shunt		
			GO:0009853	photorespiration		
			GO:0080129	proteasome core complex assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0046686	response to cadmium ion		
			GO:0051788	response to misfolded protein		
			GO:0009651	response to salt stress		
			GO:0009266	response to temperature stimulus		
			GO:0010043	response to zinc ion		
			GO:0006511	ubiquitin-dependent protein catabolic process		
			GO:0006833	water transport		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005739	mitochondrion		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0005773	vacuole		
			GO:0005507	copper ion binding		
			GO:0004807	triose-phosphate isomerase activity		
MDP0000160372	REF/SRPP-like protein At1g67360	2,0	GO:0008150	biological process	1.2e-53	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000266191	REF/SRPP-like protein At1g67360	2,0	GO:0008150	biological process	3.2e-54	AT1G67360
			GO:0005737	cytoplasm		
			GO:0005773	vacuole		
MDP0000403786	Putative serine/threonine-protein kinase receptor	2,0	GO:0005886	plasma membrane	1.7e-111	AT2G19130
			GO:0004672	protein kinase activity		

MDP0000710281	N/A	2,0	GO:0006508 GO:0009737 GO:0009414 GO:0005783 GO:0005576 GO:0070001	proteolysis response to abscisic acid response to water deprivation endoplasmic reticulum extracellular region aspartic-type peptidase activity	4.4e-19	ASPG1 ASPARTIC PROTEASE IN GUARD CELL 1
MDP0000422652	Heat shock protein STI	2,0	GO:0009165 GO:0046686 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0005634	nucleotide biosynthetic process response to cadmium ion response to high light intensity response to hydrogen peroxide cytoplasm cytosol nucleus	4.5e-229	Hop2
MDP0000795157	DnaJ homolog subfamily B member 4	2,0	GO:0006457 GO:0034976 GO:0009408 GO:0009644 GO:0042542 GO:0005737	protein folding response to endoplasmic reticulum stress response to heat response to high light intensity response to hydrogen peroxide cytoplasm	1.3e-114	AT2G20560
MDP0000190008	10 kDa chaperonin	2,0	GO:0005737 GO:0005739	cytoplasm mitochondrion	6.8e-38	AT1G23100
MDP0000608906	REF/SRPP-like protein At3g05500	2,0	GO:0006865 GO:0008150 GO:0010200 GO:0005737 GO:0005773	amino acid transport biological process response to chitin cytoplasm vacuole	3.1e-72	AT3G05500
MDP0000216106	ankyrin repeat family protein	2,0	GO:0008150 GO:0005575 GO:0003674	biological process cellular component molecular function	1.7e-75	AT5G04700
MDP0000388861	Protein-L-isoaspartate O-methyltransferase	1,9	GO:0007568 GO:0006464 GO:0009737 GO:0009651 GO:0009845 GO:0005737 GO:0005829 GO:0004719	aging cellular protein modification process response to abscisic acid response to salt stress seed germination cytoplasm cytosol protein-L-isoaspartate (D-aspartate) O-methyltransferase activity	1.9e-55	PIMT1 AT3G48330
MDP0000254930	PPPDE peptidase domain-containing protein 2	1,9	GO:0008150 GO:0005737 GO:0005634 GO:0003674	biological process cytoplasm nucleus molecular function	8.4e-90	AT3G07090
MDP0000281696	Homeobox-leucine zipper protein ATHB-54	1,9	GO:0008150 GO:0005737 GO:0008536	biological process cytoplasm Ran GTPase binding	0.089	AT1G27060
MDP0000886900	unknown	1,9	GO:0008150 GO:0003674	biological process molecular function	1.4e-48	AT5G41470
MDP0000125138	unknown protein	1,9	GO:0008150 GO:0005634 GO:0003674	biological process nucleus molecular function	1.2e-29	AT3G42800
MDP0000161691	Heat shock protein STI	1,9	GO:0009165 GO:0046686 GO:0009644 GO:0042542 GO:0005737 GO:0005829 GO:0005634 GO:0005886	nucleotide biosynthetic process response to cadmium ion response to high light intensity response to hydrogen peroxide cytoplasm cytosol nucleus plasma membrane	2.1e-213	Hop2
MDP0000152564	17.4 kDa class III	1,9	GO:0006457	protein folding	8.4e-29	AT1G54050

	heat shock protein		GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000849143	PPPDE peptidase domain-containing protein 2	1,9	GO:0008150	biological process	2.2e-89	AT3G07090
			GO:0005737	cytoplasm		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000702557	Flavonol-3-O-glycoside-7-O-glucosyltransferase 1	1,9	GO:0009507	chloroplast	1.2e-50	AT2G36780
			GO:0016757	transferase activity, transferring glycosyl groups		
			GO:0008194	UDP-glycosyltransferase activity		
MDP0000507230	Hypoxia up-regulated protein 1	1,9	GO:0030244	cellulose biosynthetic process	1.4e-302	AT4G16660
			GO:0048193	Golgi vesicle transport		
			GO:0006857	oligopeptide transport		
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009627	systemic acquired resistance		
			GO:0009507	chloroplast		
			GO:0005829	cytosol		
			GO:0005783	endoplasmic reticulum		
			GO:0005794	Golgi apparatus		
			GO:0005774	vacuolar membrane		
			GO:0008150	biological_process	0.00080	AT5G51160
			GO:0010106	cellular response to iron ion starvation		
			GO:0006826	iron ion transport		
			GO:0015706	nitrate transport		
			GO:0010167	response to nitrate		
			GO:0010043	response to zinc ion		
			GO:0005575	cellular_component		
MDP0000279217		1,9	GO:0008150	biological_process	0.00080	AT5G51160
			GO:0010106	cellular response to iron ion starvation		
			GO:0006826	iron ion transport		
			GO:0015706	nitrate transport		
			GO:0010167	response to nitrate		
			GO:0010043	response to zinc ion		
			GO:0005575	cellular_component		
MDP0000321343	Splicing factor U2af small subunit A	1,9	GO:0007067	mitosis	7.9e-91	ATU2AF35A AT1G27650
			GO:0048573	photoperiodism, flowering		
			GO:0005634	nucleus		
			GO:0003723	RNA binding		
MDP0000299388	Probable methyltransferase PMT11	1,9	GO:0005768	endosome	1.9e-265	AT2G39750
			GO:0005794	Golgi apparatus		
			GO:0005802	trans-Golgi network		
MDP0000168167	Chaperone protein ClpB 2	1,9	GO:0009658	chloroplast organization	2.9e-293	CLPB3 AT5G15450
			GO:0009408	response to heat		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0009532	plastid stroma		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
MDP0000932255	Activator of 90 kDa heat shock protein ATPase homolog 1	1,8	GO:0006457	protein folding	6.2e-134	AT3G12050
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		

			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005829	cytosol		
			GO:0005634	nucleus		
MDP0000870727	CBL-interacting serine/threonine-protein kinase 25	1,8	GO:0050832	defense response to fungus	7.2e-153	CIPK25 CBL-interacting protein kinase 25
			GO:0005886	plasma membrane		
			GO:0016301	kinase activity		
MDP0000243895	Heat shock factor protein HSF30	1,8	GO:0034605	cellular response to heat	3.9e-91	HSFA2 AT2G26150
			GO:0071456	cellular response to hypoxia		
			GO:0034620	cellular response to unfolded protein		
			GO:0010286	heat acclimation		
			GO:0045893	positive regulation of transcription, DNA-templated		
			GO:0006457	protein folding		
			GO:0010200	response to chitin		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0001666	response to hypoxia		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0005515	protein binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000226817	EGY3 (ETHYLENE-DEPENDENT GRAVITROPISM-DEFICIENT AND YELLOW-GREEN-LIKE 3)	1,8	GO:0006457	protein folding	3.8e-206	EGY3 ETHYLENE-DEPENDENT GRAVITROPISM-DEFICIENT AND YELLOW-GREEN-LIKE 3
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
MDP0000188069	Pheophorbide a oxygenase, chloroplastic	1,8	GO:0045036	protein targeting to chloroplast	7.3e-152	TIC55-II translocon at the inner envelope membrane of chloroplasts 55-II
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
MDP0000238655	Uncharacterized RNA-binding protein	1,8	GO:0043484	regulation of RNA splicing	1.2e-35	SR45a AT1G07350
			GO:0009644	response to high light intensity		
			GO:0008380	RNA splicing		
			GO:0003723	RNA binding		
MDP0000303015	Heat shock protein 101	1,8	GO:0010286	heat acclimation	0	AT1G74310
			GO:0045727	positive regulation of translation		
			GO:0006457	protein folding		
			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
MDP0000321763	Ras-related protein Rab11D	1,8	GO:0009504	cell plate	3.8e-78	RABA3 AT1G01200
			GO:0005768	endosome		
			GO:0005634	nucleus		
			GO:0005525	GTP binding		
MDP0000236662	WD repeat-containing protein 55 homolog	1,8	GO:0008150	biological_process	1.1e-77	AT3G57810
			GO:0009507	chloroplast		
			GO:0008234	cysteine-type peptidase activity		
MDP0000879589	(+)-neomenthol dehydrogenase	1,8	GO:0042335	cuticle development	2.4e-55	AT1G01800
			GO:0009409	response to cold		
			GO:0000038	very long-chain fatty acid metabolic process		
			GO:0005737	cytoplasm		

			GO:0009505	plant-type cell wall		
			GO:0016491	oxidoreductase activity		
MDP0000255592	Protein SYM1	1,8	GO:0008150	biological process	6.0e-71	AT4G33905
			GO:0009694	jasmonic acid metabolic process		
			GO:0009753	response to jasmonic acid		
			GO:0005739	mitochondrion		
			GO:0005778	peroxisomal membrane		
MDP0000217508	Heat shock protein 101	1,8	GO:0010286	heat acclimation	0	HSP101 AT1G74310
			GO:0045727	positive regulation of translation		
			GO:0006457	protein folding		
			GO:0043335	protein unfolding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
			GO:0005515	protein binding		
MDP0000614608	Probable VAMP-like protein	1,8	GO:0003674	molecular function	4.0e-44	AT5G52990
MDP0000416692	Heat shock 70 kDa protein, mitochondrial	1,8	GO:0019243	methylglyoxal catabolic process to D-lactate	5.8e-290	MTHSC70-2 mitochondrial HSO70 2
			GO:0006457	protein folding		
			GO:0046686	response to cadmium ion		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009651	response to salt stress		
			GO:0009615	response to virus		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005794	Golgi apparatus		
			GO:0005759	mitochondrial matrix		
			GO:0005739	mitochondrion		
			GO:0005774	vacuolar membrane		
			GO:0005524	ATP binding		
MDP0000322220	Heat shock cognate 70 kDa protein 1	1,8	GO:0042742	defense response to bacterium	1.7e-298	HSC70-1 heat shock cognate protein 70-1
			GO:0050832	defense response to fungus		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0010187	negative regulation of seed germination		
			GO:0006457	protein folding		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009651	response to salt stress		
			GO:0009615	response to virus		
			GO:0090332	stomatal closure		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		

			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0002020	protease binding		
			GO:0005515	protein binding		
MDP0000233640	Ribosome biogenesis protein BMS1 homolog	1,8	GO:0006406	mRNA export from nucleus	0.	AT1G06720
			GO:0006606	protein import into nucleus		
			GO:0005634	nucleus		
MDP0000282755	WD and tetratricopeptide repeats protein 1	1,8	GO:0008150	biological process	1.8e-266	ASG2 AT5G10940
			GO:0031048	chromatin silencing by small RNA		
			GO:0010413	glucuronoxylan metabolic process		
			GO:0009630	gravitropism		
			GO:0045132	meiotic chromosome segregation		
			GO:0006346	methylation-dependent chromatin silencing		
			GO:0000956	nuclear-transcribed mRNA catabolic process		
			GO:0016246	RNA interference		
			GO:0007062	sister chromatid cohesion		
			GO:0045492	xylan biosynthetic process		
			GO:0080008	Cul4-RING ubiquitin ligase complex		
			GO:0005737	cytoplasm		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0000166	nucleotide binding		
MDP0000364302		1,8	GO:0008150	biological process	5.1e-18	AT4G32208
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000260603	Pyruvate kinase isozyme G, chloroplastic	1,8	GO:0006633	fatty acid biosynthetic process	1.4e-196	PKp3 AT1G32440
			GO:0006096	glycolysis		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0000287	magnesium ion binding		
			GO:0030955	potassium ion binding		
			GO:0004743	pyruvate kinase activity		
MDP0000756448	AP-2 complex subunit mu	1,7	GO:0006886	intracellular protein transport	2.1e-15	AP2M AT5G46630
			GO:0030125	clathrin vesicle coat		
			GO:0005829	cytosol		
			GO:0005886	plasma membrane		
MDP0000708550	Chlorophyllase-2, chloroplastic	1,7	GO:0015996	chlorophyll catabolic process	1.1e-76	CLH2 chlorophyllase 2
			GO:0009507	chloroplast		
			GO:0047746	chlorophyllase activity		
MDP0000264662	Chaperone protein ClpB 2	1,7	GO:0009658	chloroplast organization	0.	CLPB3 AT5G15450
			GO:0009408	response to heat		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0009532	plastid stroma		
			GO:0005524	ATP binding		
			GO:0016887	ATPase activity		
MDP0000135570	rubredoxin family protein	1,7	GO:0009507	chloroplast	2.2e-36	AT5G51010
			GO:0046872	metal ion binding		
MDP0000274009	Probable methyltransferase PMT26	1,7	GO:0005768	endosome	2.0e-288	AT5G64030
			GO:0005794	Golgi apparatus		
			GO:0005802	trans-Golgi network		
MDP0000170311	Protein ALWAYS	1,7	GO:0008150	biological process	3.9e-216	ALY3

	EARLY 3		GO:0010413	glucuronoxylan metabolic process		ALWAYS EARLY 3
			GO:0009630	gravitropism		
			GO:0045492	xylan biosynthetic process		
			GO:0005634	nucleus		
			GO:0005515	protein binding		
MDP0000175599		1,7	GO:0008150	biological process	2.4e-07	AT2G42310
			GO:0009853	photorespiration		
			GO:0080129	proteasome core complex assembly		
			GO:0051788	response to misfolded protein		
			GO:0006511	ubiquitin-dependent protein catabolic process		
			GO:0009507	chloroplast		
			GO:0005747	mitochondrial respiratory chain complex I		
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000866655	Chlorophyll a-b binding protein 3, chloroplastic	1,7	GO:0019344	cysteine biosynthetic process	8.4e-118	LHCA3 photosystem I light harvesting complex gene 3
			GO:0015979	photosynthesis		
			GO:0009507	chloroplast		
			GO:0009534	chloroplast thylakoid		
			GO:0009535	chloroplast thylakoid membrane		
			GO:0030076	light-harvesting complex		
			GO:0016020	membrane		
			GO:0010287	plastoglobule		
			GO:0009579	thylakoid		
			GO:0016168	chlorophyll binding		
MDP0000161765	Translocase of chloroplast 159, chloroplastic	1,7	GO:0045036	protein targeting to chloroplast	3.4e-189	TOC159 translocon at the outer envelope membrane of chloroplasts 159
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0009707	chloroplast outer membrane		
			GO:0005829	cytosol		
			GO:0016020	membrane		
			GO:0009536	plastid		
			GO:0003924	GTPase activity		
			GO:0005515	protein binding		
			GO:0004888	transmembrane signaling receptor activity		
MDP0000362505	17.9 kDa class II heat shock protein	1,7	GO:0006457	protein folding	1.3e-33	HSP17.6II 17.6 kDa class II heat shock protein
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000181929	Heat shock cognate protein 80	1,7	GO:0071277	cellular response to calcium ion	8.5e-300	AT5G56010
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005886	plasma membrane		
			GO:0005524	ATP binding		
MDP0000873874	Whole genome shotgun sequence of line PN40024, scaffold_3.assembly12x	1,7	GO:0009793	embryo development ending in seed dormancy	1.8e-05	AT4G21020
			GO:0005886	plasma membrane		
MDP0000322220	Heat shock cognate 70 kDa protein 1	1,7	GO:0042742	defense response to bacterium	1.7e-298	HSC70-1
			GO:0050832	defense response to fungus		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		

			GO:0010187	negative regulation of seed germination		
			GO:0006457	protein folding		
			GO:0046686	response to cadmium ion		
			GO:0009409	response to cold		
			GO:0009408	response to heat		
			GO:0009651	response to salt stress		
			GO:0009615	response to virus		
			GO:0090332	stomatal closure		
			GO:0048046	apoplast		
			GO:0005618	cell wall		
			GO:0009507	chloroplast		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		
			GO:0005794	Golgi apparatus		
			GO:0016020	membrane		
			GO:0005730	nucleolus		
			GO:0005634	nucleus		
			GO:0005886	plasma membrane		
			GO:0009506	plasmodesma		
			GO:0005774	vacuolar membrane		
			GO:0002020	protease binding		
			GO:0005515	protein binding		
MDP0000150990	Random slug protein 5	1,7	GO:0008150	biological process	1.2e-92	AT3G22410
			GO:0005739	mitochondrion		
			GO:0003674	molecular function		
MDP0000248823	L-ascorbate peroxidase T, chloroplastic	1,7	GO:0010019	chloroplast-nucleus signaling pathway	4.3e-158	TAPX thylakoidal ascorbate peroxidase
			GO:0009631	cold acclimation		
			GO:0071588	hydrogen peroxide mediated signaling pathway		
			GO:0015979	photosynthesis		
			GO:0010027	thylakoid membrane organization		
			GO:0009507	chloroplast		
			GO:0009534	chloroplast thylakoid		
			GO:0009535	chloroplast thylakoid membrane		
			GO:0009579	thylakoid		
			GO:0016688	L-ascorbate peroxidase activity		
MDP0000614851	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	1,7	GO:0009658	chloroplast organization	1.4e-238	CPN60A chaperonin-60alpha
			GO:0009790	embryo development		
			GO:0009793	embryo development ending in seed dormancy		
			GO:0016226	iron-sulfur cluster assembly		
			GO:0048481	ovule development		
			GO:0006457	protein folding		
			GO:0048046	apoplast		
			GO:0009507	chloroplast		
			GO:0009941	chloroplast envelope		
			GO:0009570	chloroplast stroma		
			GO:0005829	cytosol		
			GO:0022626	cytosolic ribosome		
			GO:0016020	membrane		
			GO:0005739	mitochondrion		
			GO:0009579	thylakoid		
MDP0000302583	GHMP kinase family protein	1,7	GO:0042546	cell wall biogenesis	4.2e-148	GLCAK AT3G01640
			GO:0006020	inositol metabolic process		
			GO:0048868	pollen tube development		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005524	ATP binding		
			GO:0004335	galactokinase activity		
			GO:0047912	galacturonokinase activity		

			GO:0004340	glucokinase activity		
			GO:0047940	glucuronokinase activity		
			GO:0009702	L-arabinokinase activity		
			GO:0008266	poly(U) RNA binding		
MDP0000886138	Putative methyltransferase DDB_G0268948	1,7	GO:0046686	response to cadmium ion	3.8e-89	AT2G41380
			GO:0010583	response to cyclopentenone		
			GO:0009407	toxin catabolic process		
			GO:0005829	cytosol		
			GO:0005739	mitochondrion		
MDP0000184300	unknown protein	1,7	GO:0008150	biological process	3.7e-21	MAKR5 AT5G52870
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000581293	Protein CCA1	1,7	GO:0009851	auxin biosynthetic process	1.5e-52	RVE1 REVEILLE 1
			GO:0009734	auxin-activated signaling pathway		
			GO:0007623	circadian rhythm		
			GO:0048574	long-day photoperiodism, flowering		
			GO:0010600	regulation of auxin biosynthetic process		
			GO:0006355	regulation of transcription, DNA-templated		
			GO:0009737	response to abscisic acid		
			GO:0009733	response to auxin		
			GO:0009723	response to ethylene		
			GO:0009739	response to gibberellin		
			GO:0009753	response to jasmonic acid		
			GO:0009751	response to salicylic acid		
			GO:0009651	response to salt stress		
			GO:0005634	nucleus		
			GO:0003677	DNA binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000157315	Programmed cell death protein 4	1,7	GO:0008150	biological_process	2.0e-202	AT1G22730
			GO:0005634	nucleus		
			GO:0003674	molecular_function		
MDP0000778372	Calcium-activated outward-rectifying potassium channel 1	1,7	GO:0006816	calcium ion transport	3.5e-100	KCO1 AT5G55630
			GO:0030003	cellular cation homeostasis		
			GO:0030007	cellular potassium ion homeostasis		
			GO:0070838	divalent metal ion transport		
			GO:0007030	Golgi organization		
			GO:0000271	polysaccharide biosynthetic process		
			GO:0051260	protein homooligomerization		
			GO:0010029	regulation of seed germination		
			GO:0010119	regulation of stomatal movement		
			GO:0009651	response to salt stress		
			GO:0005774	vacuolar membrane		
			GO:0015269	calcium-activated potassium channel activity		
			GO:0005216	ion channel activity		
			GO:0015271	outward rectifier potassium channel activity		
MDP0000330164	Glutaredoxin-C6	1,7	GO:0048653	anther development	1.5e-38	AT4G33040
			GO:0005737	cytoplasm		
MDP0000702557	Flavonol-3-O-glycoside-7-O-glucosyltransferase 1	1,7	GO:0009507	chloroplast	1.2e-50	AT2G36780
			GO:0016757	transferase activity, transferring glycosyl groups		
			GO:0008194	UDP-glycosyltransferase activity		

Downregulated genes						
MDP0000319417	Sigma factor sigB regulation protein rsbQ	0,5	GO:0008150 GO:0005737 GO:0016787	biological process cytoplasm hydrolase activity	2.4e-74	AT3G24420
MDP0000261706	Probable serine/threonine-protein kinase At1g01540	0,3	GO:0009742 GO:0009738 GO:0019722 GO:0006468 GO:0051510 GO:0048364 GO:0009845 GO:0005886 GO:0005524 GO:0004672	brassinosteroid mediated signaling pathway abscisic acid-activated signaling pathway calcium-mediated signaling protein phosphorylation regulation of unidimensional cell growth root development seed germination plasma membrane ATP binding protein kinase activity	3.1e-164	PERK4 AT2G18470

Table 7

Transcripts differentially expressed in HT fruit compare to the control ones after 8 hours from the treatment.

MDP	Description	Ratio	Go code	GO terms	*p value*	*Arabidopsis* protein
Upregulated genes						
MDP0000209621	unknown protein	2,1	GO:0008150	biological process	7.7e-41	AT5G47830
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005739	mitochondrion		
			GO:0005634	nucleus		
			GO:0003674	molecular function		
MDP0000946614	Probable WRKY transcription factor 11	2,0	GO:0042742	defense response to bacterium	2.6e-76	WRKY11 AT4G31550
			GO:0035556	intracellular signal transduction		
			GO:0006355	regulation of transcription, DNA-templated		
			GO:0002679	respiratory burst involved in defense response		
			GO:0010200	response to chitin		
			GO:0005634	nucleus		
			GO:0005516	calmodulin binding		
			GO:0003700	sequence-specific DNA binding transcription factor activity		
MDP0000684170	Heat shock cognate 70 kDa protein 4	1,9	GO:0010286	heat acclimation	7.2e-217	heat-shock protein 70T-2
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000322755	Dihydroflavonol-4-reductase	1,8	GO:0007623	circadian rhythm	1.9e-122	CCR1 cinnamoyl coa reductase 1
			GO:0009809	lignin biosynthetic process		
			GO:0006623	protein targeting to vacuole		
			GO:0009409	response to cold		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0016621	cinnamoyl-CoA reductase activity		
MDP0000126528	F-box protein GID2	1,7	GO:0048444	floral organ morphogenesis	1.0e-42	SLY1 SLEEPY1
			GO:0009740	gibberellic acid mediated signaling pathway		
			GO:0042538	hyperosmotic salinity response		
			GO:0009867	jasmonic acid mediated signaling pathway		
			GO:0009939	positive regulation of gibberellic acid mediated signaling pathway		
			GO:0010325	raffinose family oligosaccharide biosynthetic process		
			GO:0009737	response to abscisic acid		
			GO:0009723	response to ethylene		
			GO:0009863	salicylic acid mediated signaling pathway		
			GO:0010162	seed dormancy process		
			GO:0009845	seed germination		
			GO:0005634	nucleus		
			GO:0019005	SCF ubiquitin ligase complex		
			GO:0005515	protein binding		
MDP0000256438	Small heat shock protein, chloroplastic	1,7	GO:0010286	heat acclimation	1.4e-60	HSP21 AT4G27670
			GO:0006457	protein folding		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0009507	chloroplast		
MDP0000186614	Zinc finger CCHC-type and RNA-	1,7	GO:0032502	developmental process	1.9e-61	U11/U12-31K AT3G10400
			GO:0000398	mRNA splicing, via spliceosome		
			GO:0051302	regulation of cell division		

	binding motif-containing protein 1		GO:0005634 GO:0003723	nucleus RNA binding		
MDP0000260603	Pyruvate kinase isozyme G, chloroplastic	1,7	GO:0006633 GO:0006096 GO:0009507 GO:0009570 GO:0000287 GO:0030955 GO:0004743	fatty acid biosynthetic process glycolysis chloroplast chloroplast stroma magnesium ion binding potassium ion binding pyruvate kinase activity	1.4e-196	PKp3 AT1G32440
MDP0000205113	Transcription factor bHLH62	1,7	GO:0016132 GO:0006355 GO:0005634 GO:0003677 GO:0003700	brassinosteroid biosynthetic process regulation of transcription, DNA-templated nucleus DNA binding sequence-specific DNA binding transcription factor activity	2.8e-80	AT5G48560
MDP0000614851	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	1,7	GO:0009658 GO:0009790 GO:0009793 GO:0016226 GO:0048481 GO:0006457 GO:0048046 GO:0009507 GO:0009941 GO:0009570 GO:0005829 GO:0022626 GO:0016020 GO:0005739 GO:0009579	chloroplast organization embryo development embryo development ending in seed dormancy iron-sulfur cluster assembly ovule development protein folding apoplast chloroplast chloroplast envelope chloroplast stroma cytosol cytosolic ribosome membrane mitochondrion thylakoid	1.4e-238	CPN60A chaperonin-60alpha

Down regulated genes						
MDP0000145780	Patellin-5	0,5	GO:0006810 GO:0005829 GO:0005634 GO:0005886 GO:0005215	transport cytosol nucleus plasma membrane transporter activity	1.1e-125	AT1G72160
MDP0000312731	Sec14 cytosolic factor	0,5	GO:0006810 GO:0008526 GO:0005215	transport phosphatidylinositol transporter activity transporter activity	1.1e-165	SFH12 AT4G36490
MDP0000607529	HMG1/2-like protein	0,4	GO:0006333 GO:0006096 GO:0007030 GO:0006972 GO:0046686 GO:0009651 GO:0009266 GO:0006833 GO:0000785 GO:0005634 GO:0003682 GO:0003677 GO:0003700 GO:0030527	chromatin assembly or disassembly glycolysis Golgi organization hyperosmotic response response to cadmium ion response to salt stress response to temperature stimulus water transport chromatin nucleus chromatin binding DNA binding sequence-specific DNA binding transcription factor activity structural constituent of chromatin	6.6e-34	HMGB2 high mobility group B2

Table 8

Transcripts differentially expressed in HT fruit compare to control ones after 24 hours from the treatment.

MDP	Description	Ratio	Go code	GO terms	*p value*	*Arabidopsis* protein
Upregulated genes						
MDP0000291831	17.9 kDa class II heat shock protein	2,3	GO:0006457	protein folding	1.5e-36	HSP17.6II 17.6 kDa class II heat shock protein
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000700383	17.9 kDa class II heat shock protein	2,0	GO:0006457	protein folding	8.8e-35	HSP17.6II 17.6 kDa class II heat shock protein
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0005737	cytoplasm		
MDP0000207407	18.5 kDa class I heat shock protein	1,9	GO:0010286	heat acclimation	5.3e-42	AT2G29500
			GO:0006457	protein folding		
			GO:0034976	response to endoplasmic reticulum stress		
			GO:0009408	response to heat		
			GO:0009644	response to high light intensity		
			GO:0042542	response to hydrogen peroxide		
			GO:0006979	response to oxidative stress		
			GO:0005737	cytoplasm		
			GO:0003674	molecular function		
MDP0000755113	Probable glutathione S-transferase	1,9	GO:0016036	cellular response to phosphate starvation	1.4e-85	GSTU19 AT1G78380
			GO:0042631	cellular response to water deprivation		
			GO:0006635	fatty acid beta-oxidation		
			GO:0019375	galactolipid biosynthetic process		
			GO:0006094	gluconeogenesis		
			GO:0006096	glycolysis		
			GO:0080129	proteasome core complex assembly		
			GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process		
			GO:0046686	response to cadmium ion		
			GO:0010583	response to cyclopentenone		
			GO:0051788	response to misfolded protein		
			GO:0006979	response to oxidative stress		
			GO:0009651	response to salt stress		
			GO:0009407	toxin catabolic process		
			GO:0009507	chloroplast		
			GO:0009570	chloroplast stroma		
			GO:0005737	cytoplasm		
			GO:0005829	cytosol		
			GO:0005794	Golgi apparatus		
			GO:0005886	plasma membrane		
			GO:0005774	vacuolar membrane		
			GO:0043295	glutathione binding		
			GO:0004364	glutathione transferase activity		
MDP0000154393	Cysteine-rich receptor-like protein kinase 25	1,7	GO:0006995	cellular response to nitrogen starvation	4.4e-98	CRK10 AT4G23180
			GO:0005886	plasma membrane		
			GO:0016301	kinase activity		
MDP0000681724	Expansin-A1	1,7	GO:0009828	plant-type cell wall loosening	9.2e-102	EXPA10 expansin A10
			GO:0009831	plant-type cell wall modification involved in multidimensional cell growth		
			GO:0006949	syncytium formation		
			GO:0009826	unidimensional cell growth		
			GO:0005576	extracellular region		

			GO:0009505 GO:0005199	plant-type cell wall structural constituent of cell wall		
MDP0000216946	Probable NADP-dependent oxidoreductase P2	1,7	GO:0030036 GO:0030048 GO:0009827 GO:0009860 GO:0005737	actin cytoskeleton organization actin filament-based movement plant-type cell wall modification pollen tube growth cytoplasm	4.4e-132	AT3G03080

Table 9

Transcripts commonly regulated after 30 minutes and 1 hour in HT and Control fruits, and qRT-PCR analysis

MDP	Description	Microarray		qRT-PCR data	
Upregulated genes		**Ratio 30**	**Ratio 1**	**Ratio 30**	**Ratio 1**
MDP0000684170	Heat shock cognate 70 kDa protein 4	3,1	3,5		
MDP0000657396	Protein FAM32A	3,0	4,2		
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	2,6	2,3		
MDP0000220754	unknown protein	2,6	2,0		
MDP0000290546	DnaJ homolog subfamily B member 13	2,5	3,3		
MDP0000456824	Chaperone protein dnaJ 11, chloroplastic	2,5	2,1		
MDP0000543224	Probable pyridoxal biosynthesis protein PDX1.2	2,5	4,8		
MDP0000145643	REF/SRPP-like protein At1g67360	2,4	3,0		
MDP0000153978	ATBAG5 (arabidopsis thaliana bcl-2-associated athanogene 5); protein binding	2,4	2,5		
MDP0000209621	unknown protein	2,4	3,8		
MDP0000235765	Chaperonin CPN60-2, mitochondrial	2,4	2,3		
MDP0000161691	Heat shock protein STI	2,4	1,9	8,2+E00±1,2	1,6+E01±3,5
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	2,3	2,7		
MDP0000172863	Probable inactive receptor kinase At1g27190	2,3	2,2		
MDP0000225534	Purple acid phosphatase 23	2,3	2,6		
MDP0000254930	PPPDE peptidase domain-containing protein 2	2,3	2,9		
MDP0000311339	Heat shock 70 kDa protein	2,3	3,4		
MDP0000872073	Putative uncharacterized protein	2,3	2,3		
MDP0000878181	unknown protein	2,3	1,9		
MDP0000290618	Peroxisome biogenesis factor 10	2,2	2,8		
MDP0000302297	Somatic embryogenesis receptor kinase 1	2,2	3,1		
MDP0000744777	Protein popC	2,2	2,8		
MDP0000122734	Heat shock cognate 70 kDa protein 4	2,1	2,6		
MDP0000164489	18.5 kDa class I heat shock protein	2,1	2,9		
MDP0000256438	Small heat shock protein, chloroplastic	2,1	2,0		
MDP0000438504	Phospholipase C 3	2,1	2,7		
MDP0000440811	Cyclin-D3-2	2,1	1,8		
MDP0000707429	transferase, transferring glycosyl groups	2,1	2,8		
MDP0000795157	DnaJ homolog subfamily B member 4	2,1	2,6		
MDP0000160372	REF/SRPP-like protein At1g67360	2,0	2,4		
MDP0000160372	REF/SRPP-like protein At1g67360	2,0	2,5		
MDP0000205027	Putative uncharacterized protein	2,0	2,1		
MDP0000207407	18.5 kDa class I heat shock protein	2,0	2,9	1,1+E01±5,5	2,4+E01±9,6
MDP0000243895	Heat shock factor protein HSF30	2,0	1,7		
MDP0000271554	HSA32 (HEAT-STRESS-ASSOCIATED 32); catalytic	2,0	3,1		
MDP0000275263	THO complex subunit 4	2,0	2,4		
MDP0000303430	Heat shock protein 83	2,0	2,6		
MDP0000314161	Cyclin-A2-3	2,0	2,5		
MDP0000322220	Heat shock cognate 70 kDa protein 1	2,0	2,1		
MDP0000446336	Pentatricopeptide repeat-containing protein At4g38150	2,0	2,3		
MDP0000574524	17.5 kDa class I heat shock protein	2,0	2,5		
MDP0000768256	Hsp70-binding protein 1	2,0	2,6		
MDP0000849143	PPPDE peptidase domain-containing protein 2	2,0	2,4		
MDP0000920394	unknown protein	2,0	1,6		
MDP0000125300	Small heat shock protein, chloroplastic	1,9	3,2		
MDP0000203303	N/A	1,9	2,4		
MDP0000214382	25.3 kDa heat shock protein, chloroplastic	1,9	2,3		
MDP0000226817	EGY3 (ethylene-dependent gravitropism-deficient and yellow-green-like 3)	1,9	1,9		
MDP0000226817	EGY3 (ethylene-dependent gravitropism-deficient and yellow-green-like 3)	1,9	1,9		
MDP0000243895	Heat shock factor protein HSF30	1,9	2,4		
MDP0000285549	Uncharacterized protein At4g08330, chloroplastic	1,9	2,0		

MDP0000304623	Chaperone protein ClpB 2	1,9	1,7		
MDP0000465593	E3 ubiquitin-protein ligase RING1-like	1,9	2,2		
MDP0000508644	unknown protein	1,9	3,1		
MDP0000575835	Uncharacterized calcium-binding protein At1g02270	1,9	1,8		
MDP0000755970	Heat shock protein 101	1,9	2,7		
MDP0000807190	ABC transporter B family member 26, chloroplastic	1,9	1,7		
MDP0000145643	REF/SRPP-like protein At1g67360	1,8	2,0		
MDP0000160372	REF/SRPP-like protein At1g67360	1,8	2,4		
MDP0000171695	unknown protein	1,8	3,6		
MDP0000181929	Heat shock cognate protein 80	1,8	2,0		
MDP0000202749	THO complex subunit 4	1,8	2,1		
MDP0000254930	PPPDE peptidase domain-containing protein 2	1,8	3,2		
MDP0000266191	REF/SRPP-like protein At1g67360	1,8	2,4		
MDP0000446914	Glycogenin-1	1,8	2,6		
MDP0000842179	Granule-bound starch synthase 2, chloroplastic/amyloplastic	1,8	1,2	1,6+E00±0,4	3,2+E00±0,5
MDP0000878773	1-aminocyclopropane-1-carboxylate oxidase homolog 1	1,8	2,8	9,2+E00±2,1	1,9+E01±2,1
MDP0000152242	Triosephosphate isomerase, cytosolic	1,7	2,1		
MDP0000168932	Cysteine proteinase RD21a	1,7	2,0		
MDP0000184300	unknown protein	1,7	2,1		
MDP0000186561	Peptidyl-prolyl cis-trans isomerase cyp5	1,7	1,4		
MDP0000205027	Putative uncharacterized protein	1,7	3,1		
MDP0000209143	Uncharacterized protein R707	1,7	1,9		
MDP0000217508	Heat shock protein 101	1,7	2,6		
MDP0000264662	Chaperone protein ClpB 2	1,7	1,8		
MDP0000549793	ATERDJ3A; oxidoreductase	1,7	3,0		
MDP0000614608	Probable VAMP-like protein At1g33475	1,7	1,6		
MDP0000849143	PPPDE peptidase domain-containing protein 2	1,7	2,9		
MDP0000119199	Heat stress transcription factor A-6b	1,6	1,8		
MDP0000150162	20 kDa chaperonin, chloroplastic	1,6	1,9		
MDP0000150990	Random slug protein 5	1,6	2,3		
MDP0000176947	Probable LRR receptor-like serine/threonine-protein kinase	1,6	1,7		
MDP0000206753	Putative pentatricopeptide repeat-containing protein At5g40405	1,6	1,3		
MDP0000232642	Protein translation factor SUI1 homolog	1,6	1,7		
MDP0000235822	[Protein-PII] uridylyltransferase	1,6	1,6		
MDP0000238655	Uncharacterized RNA-binding protein C25G10.01	1,6	2,4		
MDP0000303015	Heat shock protein 101	1,6	2,9		
MDP0000303430	Heat shock protein 83	1,6	2,4		
MDP0000322220	Heat shock cognate 70 kDa protein 1	1,6	1,8		
MDP0000322220	Heat shock cognate 70 kDa protein 1	1,6	1,7		
MDP0000407903	unknown protein	1,6	1,3		
MDP0000416692	Heat shock 70 kDa protein, mitochondrial	1,6	1,6		
MDP0000879589	(+)-neomenthol dehydrogenase	1,6	1,9		
MDP0000921426	integral membrane transporter family protein	1,6	1,8		
MDP0000168167	Chaperone protein ClpB 2	1,5	1,6		
MDP0000190008	10 kDa chaperonin	1,5	2,2		
MDP0000208958	15.7 kDa heat shock protein, peroxisomal	1,5	1,7		
MDP0000255483	UPF0481 protein At3g47200	1,5	1,6		
MDP0000283650	Peroxidase 4	1,5	1,5		
MDP0000295085	Ubiquitin fusion degradation protein 1 homolog	1,5	2,2		
MDP0000295392	CBL-interacting serine/threonine-protein kinase 21	1,5	1,3		
MDP0000298185	Pentatricopeptide repeat-containing protein At1g79080, chloroplastic	1,5	1,8		
MDP0000302471	lactoylglutathione lyase family protein / glyoxalase I family protein	1,5	1,2		
MDP0000321529	FLA21 (fasciclin-like arabinogalactan protein 21 precursor)	1,5	1,4		
MDP0000466683	Glycogenin-1	1,5	2,8	1,8+E01±2,6	2,5+E01±11,8
MDP0000563240	Polyadenylate-binding protein-interacting protein 2	1,5	2,3		
MDP0000622085	Probable sodium-coupled neutral amino acid transporter 6	1,5	1,5		
MDP0000757565	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	1,5	1,2		
MDP0000875479	unknown protein	1,5	1,9		
MDP0000930889	Polyubiquitin	1,5	2,2		
MDP0000125594	Uncharacterized protein C594.04c	1,4	1,4		

MDP0000192625	Putative serine/threonine-protein kinase receptor	1,4	1,7		
MDP0000212717	Probable LRR receptor-like serine/threonine-protein kinase	1,4	1,5		
MDP0000231034	Pre-mRNA-splicing factor SF2	1,4	1,4		
MDP0000236662	WD repeat-containing protein 55 homolog	1,4	2,3		
MDP0000316093	Whole genome shotgun sequence of line PN40024, scaffold_71.assembly12x	1,4	2,0		
MDP0000542297		1,4	1,5		
MDP0000581293	Protein CCA1	1,4	1,6		
MDP0000655215	1-acyl-sn-glycerol-3-phosphate acyltransferase 1, chloroplastic	1,4	1,2		
MDP0000755113	Probable glutathione S-transferase	1,4	2,9		
MDP0000230007	97 kDa heat shock protein	1,3	1,8		
MDP0000252890	Ribulose bisphosphate carboxylase small chain, chloroplastic	1,3	1,3		
MDP0000614851	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	1,3	2,0		
MDP0000833759	DnaJ protein homolog	1,2	1,7		
MDP0000871896	Retinol dehydrogenase 11	1,2	1,5		
MDP0000220526	Aspartate-semialdehyde dehydrogenase	0,6	1,2	1,0+E00±0,1	1,2+E00±0,1
Down regulated genes					
MDP0000174729	unknown protein	0,4	0,4		
MDP0000906115	Flavonol synthase/flavanone 3-hydroxylase	0,5	0,7		
MDP0000323212	Auxin-repressed 12.5 kDa protein	0,5	0,6		
MDP0000334850	wound-responsive protein-related	0,5	0,5		
MDP0000383777	Amino acid permease 3	0,5	0,7		
MDP0000939633	AP2/ERF and B3 domain-containing transcription repressor TEM1	0,5	0,5		
MDP0000240840	Ethylene-responsive transcription factor 5	0,5	0,6		
MDP0000276302	SH3 domain-containing protein	0,6	2,1		
MDP0000124555	Transcription factor WER	0,6	0,7		
MDP0000135540	Homeobox-leucine zipper protein HAT14	0,6	0,6	5,9+E-01±0,2	5,0+E-01±0,2
MDP0000137498	OXS3 (oxidative stress 3)	0,6	0,7		
MDP0000138306	Polyphosphoinositide phosphatase	0,6	0,7		
MDP0000139075	Phenylalanine ammonia-lyase 1	0,6	0,6		
MDP0000139367	Serine/threonine-protein kinase fray2	0,6	0,8		
MDP0000165187	Peptide transporter PTR5	0,6	0,7		
MDP0000179053	Putative uncharacterized protein	0,6	0,7		
MDP0000949591	unknown protein	0,6	0,6		
MDP0000649644	unknown protein	0,6	0,5		
MDP0000480599	N/A	0,6	0,6		
MDP0000425402	Transmembrane protein 131	0,6	0,5		
MDP0000362286	Auxin-repressed 12.5 kDa protein	0,6	0,5		
MDP0000309314	Probable mitochondrial chaperone bcs1	0,6	0,6		
MDP0000297569	Beta-glucosidase 24	0,6	0,7		
MDP0000292277	Predicted protein	0,6	0,5		
MDP0000219591	26S proteasome non-ATPase regulatory subunit 12	0,6	0,8		
MDP0000047589	Beta-glucosidase 12	0,6	0,5		
MDP0000125070	Proliferating cell nuclear antigen	0,7	0,7		
MDP0000180890	Tyrosine aminotransferase	0,7	0,6	9,3+E-01±0,1	6,6+E-01±0,2
MDP0000192151	S-linalool synthase	0,7	0,6		
MDP0000943290	Predicted protein	0,7	0,7		
MDP0000769354	Zinc finger protein 1	0,7	0,7		
MDP0000804427	harpin-induced protein-related / HIN1-related / harpin-responsive protein-related	0,7	0,6		
MDP0000750392	Auxin response factor 18	0,7	0,6		
MDP0000830926	Probable gibberellin receptor GID1L3	0,7	0,5		
MDP0000565690	Protein TIFY 11B	0,7	0,4		
MDP0000490749	Probable glutathione S-transferase	0,7	0,7		
MDP0000388769	Phenylalanine ammonia-lyase 1	0,7	0,7	5,3+E-01±0,3	5,8+E-01±0,2
MDP0000315078	NADPH--cytochrome P450 reductase	0,7	0,6		
MDP0000196867	Probable pectinesterase/pectinesterase inhibitor 51	0,7	0,8		
MDP0000256258	StAR-related lipid transfer protein 7, mitochondrial	0,7	0,7		
MDP0000460903	U-box domain-containing protein 6	0,7	0,8		
MDP0000221451	Nucleobase-ascorbate transporter 3	0,7	0,7		
MDP0000222555	Chaperone protein dnaJ 8, chloroplastic	0,7	0,6		

MDP0000478657	F-box protein At5g39450	0,8	1,4		
MDP0000178949	SWI/SNF complex component SNF12 homolog	0,8	0,8		
MDP0000334150	Uncharacterized protein At3g27210	0,8	0,7		
MDP0000273751	Phytochrome A-associated F-box protein	0,8	0,7		
MDP0000266132	ATPase 11, plasma membrane-type	0,8	0,8		
MDP0000253076	Uncharacterized protein At1g15400	0,8	0,7		
MDP0000221668	Insulin-degrading enzyme	0,8	0,7		
MDP0000344348	BES1/BZR1 homolog protein 4	0,9	0,7		
MDP0000543718	Auxin-responsive protein IAA29	0,9	0,6		

Table 10

Transcripts induced in HT samples compared to control one from 15 minutes to 4 hours from the heat treatment.

MDP	Description	Ratio 15	Ratio 30	Ratio 1	Ratio 4
Upregulated genes					
MDP0000543224	Probable pyridoxal biosynthesis protein PDX1.2	3.3	2.5	4.8	2.0
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	3.2	3.3	3.7	2.1
MDP0000290546	DnaJ homolog subfamily B member 13	3.1	2.5	3.3	2.5
MDP0000684170	Heat shock cognate 70 kDa protein 4	2.9	3.1	3.5	2.4
MDP0000707429	transferase, transferring glycosyl groups	2.8	2.1	2.8	2.0
MDP0000145643	REF/SRPP-like protein At1g67360	2.6	2.5	3.0	2.4
MDP0000302297	Somatic embryogenesis receptor kinase 1	2.5	2.2	3.1	3.1
MDP0000207407	18.5 kDa class I heat shock protein	2.4	2.0	2.9	2.3
MDP0000303015	Heat shock protein 101	2.4	1.6	2.9	1.8
MDP0000657396	Protein FAM32A	2.3	3.0	4.2	2.6
MDP0000311339	Heat shock 70 kDa protein	2.3	2.3	3.4	2.5
MDP0000574524	17.5 kDa class I heat shock protein	2.3	2.0	2.5	2.2
MDP0000314161	Cyclin-A2-3	2.3	2.0	2.5	2.2
MDP0000226817	EGY3	2.3	1.9	1.9	1.8
MDP0000466683	Glycogenin-1	2.2	1.5	2.8	2.0
MDP0000150261	Conserved oligomeric Golgi complex subunit 7	2.1	2.6	3.7	2.3
MDP0000122734	Heat shock cognate 70 kDa protein 4	2.1	2.1	2.3	2.0
MDP0000171695	unknown protein	2.1	1.8	3.6	2.0
MDP0000161691	Heat shock protein STI	2.0	2.4	1.9	1.9
MDP0000275263	THO complex subunit 4	2.0	2.0	2.4	2.0
MDP0000465593	E3 ubiquitin-protein ligase RING1-like	2.0	1.9	2.2	1.6
MDP0000243895	Heat shock factor protein HSF30	2.0	1.7	2.7	1.8
MDP0000744777	Protein popC	1.9	2.2	2.8	1.4
MDP0000795157	DnaJ homolog subfamily B member 4	1.9	2.1	2.6	2.0
MDP0000768256	Hsp70-binding protein 1	1.8	2.0	2.6	2.1
MDP0000446914	Glycogenin-1	1.8	1.8	2.6	2.1
MDP0000271554	HSA32 (heat-stress-associated 32); catalytic	1.7	2.0	3.1	2.6
MDP0000205027	Putative uncharacterized protein	1.7	2.0	2.1	1.5
MDP0000205027	Putative uncharacterized protein	1.7	1.7	3.1	1.6
MDP0000549793	ATERDJ3A; oxidoreductase	1.7	1.7	3.0	2.1
MDP0000184300	unknown protein	1.6	1.7	2.1	1.7
MDP0000238655	Uncharacterized RNA-binding protein C25G10.01	1.6	1.6	2.3	1.8
MDP0000160372	REF/SRPP-like protein At1g67360	1.5	1.8	2.4	2.2

The DA meter, a dynamic tool for rapid evaluation of peach shelf-life: a preliminary study

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6.0 Abstract

The relationship between the Index of Absorbance Difference (I_{AD}) and Monilinia rot susceptibility was studied in two varieties of peach fruit ('Springbelle' and 'Redhaven'), before storage. Fruits, were divided in two lots and each lot was divided in two I_{AD} classes: class I, I_{AD}: 0.2 -0.4 and class II, I_{AD}: 0.5-0.9 for 'Springbelle' , and class I, I_{AD}: 0.0-0.3 and class II, I_{AD}: 0.4 -0.6 for 'Redhaven' . Fruits of the first lot were artificially inoculated with a conidia suspension of *Monilinia fructicola* (10^3 conidia per ml) and stored at 20°C for 3 d. Fruits of the second lot were evaluated for natural infections of *Monilinia* spp. after 6 d at 20°C. The ethylene emission of five to ten fruits per I_{AD} class was assessed before storage. In addition, flesh firmness (FF), soluble solid content (SSC) and titratable acidity (TA) were measured on a sample of 25 fruits for each variety and I_{AD} class at harvest, 3 and 6 days of storage at 20°C. In fruits artificially inoculated, the severity of disease was significantly higher in peaches in class I (more ripe) than in class II (less ripe). A similar behaviour was observed in fruits naturally infected; the percentage of infected fruit was significantly higher in class I (60% and 61.3% for 'Springbelle' and 'Redhaven' peaches respectively) than in class II (10% and 1.3% for 'Springbelle' and 'Redhaven' peaches respectively). The ethylene production of the I_{AD} classes was significantly different at harvest and probably, plays an important role in pathogen development. The results indicate that the I_{AD}can be regarded as an "easy to use" parameter to select peaches in view their susceptibility to brown rot during shelf-life.

Keywords: Index of absorbance difference; Brown rot; *Prunus persica* (L.) Batsh; *Monilinia fructicola*; Shelf-life; Ethylene

6.1 Introduction

Brown rot caused by *Monilinia laxa* (Aderhold and Ruhland) Honey, *Monilinia fructicola* (Winter) Honey and *Monilinia fructigena* (Aderhold and Ruhland) is economically an important disease in warm, humid climate areas where stone fruits are cultivated. The pathogens infect the fruit in the field and remain quiescent until they reach maturity, allowing *Monilinia* to overcome host defences (Emery, et al., 2000) and produce the disease; these infections are often classified as "quiescent" or "latent" according to how the infection is initiated (Prusky, 1996). Monilinia rot occurring in the postharvest phase, during storage and transport can be typically more severe than in the preharvest phase, reaching high values (59%) (Larena et al. 2005). In addition to these direct losses, indirect losses, deriving from the appearance of disease after fruit purchase, at consumer level, can negatively affect consumer satisfaction and propensity to consumption. The control of *Monilinia* rot depends on an integrated strategy based on cultural practices, orchard fungicide treatments and, after harvest, on the maintenance of proper storage conditions in the packinghouse and during commercialization; however, in certain years with high disease levels this strategy can be ineffective. Furthermore, no chemical treatments are allowed on stone fruit after harvest in European countries (Jemric et al. 2011) when the risk of rot appearance and drop in value of fruit is more realistic. Therefore, early, rapid, sensitive and reliable methods for assessment of a probable incidence of Monilinia rot at harvest become crucial for timely and effective fruit management. Moreover, it could help in selecting the lowest contaminated lots to be sent to long-time storage or to distant markets. In previous works, several models have been developed to obtain reliable information on the presence of pathogens on asymptomatic fruits. These models based on microbiological, molecular and physical methods have the main aim of predicting fungal infections before harvest in order to improve the timing and the efficacy of fungicide treatments; they were performed on *Botrytis cinerea* of table grape, (Broome et al. 1995; Sanzani et al. 2012) and of kiwifruit (Michailides and Morgan, 1996); on *Monilinia* spp. of sweet cherries (Förster and Adaskaveg, 2000), and on *Penicillium digitatum* of citrus fruit (Lorente et al. 2013). Nevertheless, there is a need for a model that, by integrating the biological and chemical properties of fruit, can estimate in advance the risk of deterioration in storage.In the last few years, the interest of researchers in the development of non-destructive techniques to accurately evaluate fruit ripening and assess internal quality attributes has increased. Among these non-destructive approaches, visible/near infrared (vis/NIR) spectroscopy seems particularly promising since it provides fast and reliable information on the internal characteristics of many fruit species, including stone fruit (Vanoli and Buccheri, 2012). One of these devices, the DA-Meter, measures the new ripening Index of Absorbance

Difference (I_{AD}), that correlates with the actual flesh chlorophyll-α content and fruit ethylene production (Ziosi et al., 2008). This relationship is variety specific, associated with consumer acceptability and highly reliable (Ziosi et al., 2008) for yellow fleshed peaches and nectarines. The I_{AD} is more correlated with fruit ripeness and more reliable than the physico-chemical variables commonly used to describe the maturation process (Costa et al. 2009). In the present study, an I_{AD} assay was performed to estimate, before storage, the possible presence of Monilinia rot in asymptomatic harvested peach fruit. For this purpose the correlations betweenI_{AD} and i) fruit chlorophyll content and ethylene emission, and ii) brown rot incidence in naturally and artificially infected peaches were investigated.

6.2 Materials and Methods

6.2.1 Fruit

Peach [*Prunus persica* (L.) Batsh cv 'Springbelle' and 'Redhaven'] fruits were cultivated in the experimental orchard of the Department of Agricultural Science, located in Bologna, (Italy) (coordinates 44.559592°-11.410246°). The orchard was under conventional management but no fungicide treatments against *Monilinia* spp. were carried out. Fruits harvested at commercial maturity, free of visible wounds and rot and homogeneous in size were immediately processed for I_{AD} classification.

6.2.2 I_{AD} classification and fruit analysis

Peach fruits derived from each variety were divided in two lots and each fruit was measured using a portable DA-Meter (TR-Turoni, Forlì, Italy), that gives the ripening I_{AD}. In order to define the ripening classes for the experiment, ethylene emission of five to ten fruit per I_{AD} class was assessed before storage, as described by Ziosi et al. (2008). The ethylene production was measured by placing the whole fruit in a 1 L jar sealed with an air-tight lid equipped with a rubber stopper, and left at room temperature for 1 h. A 10 mL gas sample was taken from the jar and injected into a Dani HT 86.01 (Dani, Milan, Italy) packed-gas chromatograph as described previously by Bregoli et al. (2002). After ethylene measurement, each fruit lot was divided in two I_{AD} classes: specifically, class I: 0.2 -0.4 (climacteric peak) and class II: 0.5-0.9 (onset of climacteric) for 'Springbelle' and class I: 0.0-0.3 (climacteric peak) and class II: 0.4 -0.6 (onset of climacteric) for 'Redhaven' peaches. In addition, flesh firmness (FF), soluble solid content (SSC) and titratable acidity (TA) were measured on a sample of 25 fruits for each variety and I_{AD} class. The FF was evaluated on the two opposite sides of each fruit, after eliminating a thin layer of the epicarp, using an automatic pressure tester (FTA-GUSS, South Africa) fitted with an 8 mm plunger. The SSC was determined with an Atago digital refractometer (Optolab, Modena, Italy) by squeezing a part of the mesocarp. The TA

was determined on 20mL of flesh juice (titration with 0.25NNaOH) using a semiautomatic instrument (Compact-S Titrator, Crison, Modena, Italy). The same measurements were also performed twice during shelf-life (20°C) at three (S3) and six (S6) days after harvest.

6.2.3 Pathogen

M. fructicola isolate was obtained from our collection, previously identified by sequencing of ribosomal DNA ITS regions (Mari et al., 2012) and maintained on potato dextrose agar (PDA) at 4 °C until use. In order to obtain a good sporulation, the pathogen was inoculated on V-8 agar (V8A: 250 ml of pure V8 juice and 40 g of agar in 1 L of distilled water) and incubated at 25°C with 12 h dark, 12 h light cycles for 10 days. Conidia suspensions were prepared by washing the colonies with sterile distilled water containing 0.05% (v/v) of Tween 80, quantified with a haemocytometer and diluted to the concentration of 10^3 conidia per ml.

6.2.4 Influence of I_{AD} on Monilinia rot

Artificially infected fruit. Fruits of the first lot, previously classified in two I_{AD} classes,as reported above, were wounded with a sterile nail (2x2x2mm) and inoculated with 20 µl of the *M. fructicola* conidia suspension adjusted to 10^3 mL^{-1}. Fruits were stored at 20°C for 3 d and successfully evaluated for lesion diameter. The sample unit was represented by 4 replicates of 20 fruits each for eachclass and each variety.

Naturally infected fruit. Fruits of the second lot, previously classified by I_{AD} as described above, were stored at 20°C for 6 d; the natural disease incidence was then recorded. The sample unit was represented by 4 replicates of 20 fruits for eachI_{AD} and variety.

6.2.5 Data Analysis

Data were subjected to one-way analysis of variance (ANOVA) using the statistical package Statistica for Windows (Statsoft Inc.). Separation of means was performed using the least significance difference (LSD) test at $P< 0.05$. All experiments were carried out in a completely randomized block design.

6.3 Results and Discussion

6.3.1 I_{AD} classification and fruit analysis

The *IAD* showed an high capacity for determining the harvest date and sorting stone fruit according to their ripeness, (Infante et al., 2012), but only few results were available on use of the *IAD* as field ripening predictor index (Reig et al., 2012).Budde et al. (2000) studied the time-course of ethylene evolution in relation to softening and ground color changes in peach fruit of the varieties ‘Red Globe’ and ‘July Elberta’ during the shelf life. Their results showed that statistical significant differences between ethylene amounts were observed only if the

measurements were performed every two-three days and not daily. Similar results were also obtained in a preliminary experiment with nectarine fruit of the variety 'California' (data not showed). Several authors also described that fruit harvested with similar IAD Index at harvest maintained the same behavior in term of softening, length of the shelf life as well as consumer satisfaction during the post-harvest (Ziosi et al., 2008; Reig et al., 2012; Infante, 2012; Lurie et al., 2013; Shinya et al., 2013). In the present work, defined on the basis of fruit ethylene emission, two ripening classes were considered for each variety under this study (Table 1). The ethylene production of the two I_{AD} classes was significantly different at harvest (Table 1) and was maintained or amplified during shelf-life (Figure 1 a,b), for each variety. These results confirmed the strong relationship between peach ripening stage (expressed as I_{AD}) and ethylene emission, highlighting the role of the I_{AD} as a sensitive non-destructive marker to cluster fruit into homogeneous groups (Ziosi et al., 2008; Bonora et al., 2013 a). Even considering the traditional quality traits, significant differences were observed comparing SSC, TA (Table 2) and FF (Figure 2 a,b) of the two ripening classes for each variety at harvest. The greatest differences in particular characterized the early variety 'Springbelle'. Other authors described the I_{AD} as an index having a high correlation with the most common parameters used for monitoring ripening, especially FF (Reig et al., 2012; Infante, 2012; Bonora et al., 2013 b), while SSC is considered as ethylene-independent and the researchers often did not observe marked differences in SSC between ripening stages (Ziosi et al., 2008; Hale et al., 2012; Bonora et al., 2013 b). After harvest, fruits of both varieties linearly increased their ethylene production, maintaining the differences between ripening classes, with the exception of the more ripe class (I_{AD} 0.2-0.4) of the 'Springbelle' variety, that reached the plateau 3 d after harvest (Fig. 1 a, b). Similar results had already been observed for a late nectarine, 'California', in which fruits grouped in commercial ripening class (onset of climacteric) and immature class (before climacteric) at harvest maintained the ethylene production differences over the shelf life period, while fruits of the more ripe class (climacteric peak) reached the plateau after 4 d of shelf life (data not shown). After 3 d of shelf-life, the fruit TA decreased in both varieties, while a constant SSC was observed, even if the differences existing between ripening classes were maintained up to 6 d after harvest (Table 2). Conversely, Aly et al., (1981) showed an increase in the SSC two weeks after harvest on 'Flordagold' peaches, probably due to the longer storage period and the fruit water losses. Considering FF variations, all the fruits of both varieties decreased below 1 kg/cm^2 after 3 d of shelf life, with the exception of the less ripe class of the early 'Springbelle' variety (I_{AD} 0.5-0.9), that reached edible firmness 6 d after harvest (Fig. 2 a,b). Our findings confirmed that fruit ripening and softening are two synchronized processes. Other authors observed a faster softening during the shelf life of peach fruit characterized by a

low I_{AD} value at harvest (more ripe), if compared with less ripe fruit (Lurie et al., 2013). Shynia et al. (2013) suggested that establishing the optimal harvest time in peach is a crucial issue because it regulates the softening rate to a great extent during the postharvest period and thereby determines the potential shelf life of the fruit.

6.3.2 Influence of I_{AD} on Monilinia rot

The influence of I_{AD} on Monilinia rot was tested on fruit inoculated with *M. fructicola* and naturally infected. All fruits inoculated with *M. fructicola* were rotted (data not shown), while fruits classified in two different classes of I_{AD}, class I more ripe than class II, revealed a diverse susceptibility to the pathogen. In fruit artificially inoculated, the severity of disease was significantly higher in peaches of class I than those of class II; the lesion diameters ranged from 10.1 mm ('Springbelle') to 13 mm ('Redhaven) in class I, and from 7.5 mm ('Springbelle') to 9 mm ('Redhaven') in class II (Table 3). A similar behaviour was observed in fruit naturally infected; in fact the percentage of infected fruit was significantly higher in class I (60% and 61.3% for 'Springbelle' and 'Redhaven' peaches respectively) than in class II (10% and 1.3% for 'Springbelle' and 'Redhaven' peaches respectively). *Monilinia* spp. proves to be a seriously damaging pathogen for stone fruits, particularly after harvest. In fact, fruit apparently sound at harvest, after 3 d at 20°C, showed a high incidence of brown rot, more than 50% in 'Springbelle' and 'Readhaven' peaches (Table 3).

As reported in the paragraph on I_{AD} classification and fruit analysis, a great difference was found in ethylene production between the two I_{AD} classes in both varieties. In the past, the possible involvement of ethylene in plant pathogenesis has attracted the attention of many investigators with contradictory considerations (Archer and Hislop, 1975; El-Kzzaz et al., 1983; Palou et al., 2003). A significant stimulation of spore germination and germ tube length of *M. fructicola* were found after exposure to 1 and 10 µl /L of exogenous ethylene for 6 and 24 h respectively in *in vitro* trials, while higher concentrations (100 and 1,000 µl /L) of gas did not affect either parameter. In addition, the treatment had no influence on growth rate of the fungus on potato dextrose agar (El-Kzzaz et al., 1983). In contrast, treating stone fruit with increasing concentrations (1; 10; 100 µl /L) of exogenous ethylene, Palou et al. (2003) stated the lack of influence on both incidence and severity of brown rot caused by *M. fructicola* on 'Elegant Lady' peach during cold storage. It is known that diseased plant tissue produces more ethylene than healthy tissue (Archer and Hislop, 1975) and different postharvest pathogens such as *Penicillium digitatum* (Chalutz et al., 1977) or *B. cinerea* (Qadir et al., 1997) produce ethylene. Methionine is a precursor of ethylene and since methionine is present in higher plants it is difficult to determine if the ethylene produced is of pathogen or host origin. However, in our experiments, the ethylene detected in peaches at harvest probably derived from the fruits, as

they were asymptomatic. Since the gas concentrations revealed in fruit classified in class I were very close to those found stimulating *M. fructicola* (2.88 and 1.15 µl/L for 'Springbelle' and 'Readhaven' respectively), ethylene could be partially responsible forthe high susceptibility to the pathogen of the fruit classified in class I. More investigations are in progress to evaluate the actual role of ethylene in spore germination and germ tube length.

The efficacy of *Monilinia* spp. control strategies is greatly compromised by the ability of this kind of causal agent that, like other pathogens such as *B. cinerea* (Elmer and Michailides, 2004), remain latent in the host tissue until ripening. The fruit susceptibility to infections rises in relation to the increase in sugar and decrease in the content of defensive secondary metabolites. In our experiments, fruit of class I showed a significantly higher SSC than that reported for class II; as SSC is represented substantially by sugars, their considerable presence in the peaches of class I could contribute to disease development. On the other hand, in 'Springbelle' peaches a significantly higher TA value, expressed as malic acid, was found in class II (11.2 g/L) than in class I (9.8 g/L) and this difference was maintained during shelf-life. These data are in agreement with those reported by Ziosi et al. (2008) on 'Star Red Gold', 'Laura' and 'Fayette' fruit. The hypothesis that malate, one of the main organic acids in peach fruit, can influence the disease appearance is under investigation. Preliminary *in vitro* results revealed a growth inhibition of several postharvest pathogens, included *M. laxa*, due to malic acid assayed at juice fruit concentrations (Spadoni et al. not published).

In peach, harvest maturity is usually based on fruit colour and size (Eccher Zerbini et al., 1994); however, this visual assessment does not provide a fully reliable identification of the fruit ripening stage and apparently similar fruit can show different maturity stages, influencing the appearance of postharvest *Monilinia* spp. infections. Fruit classified in two different classes of I_{AD} revealed a diverse susceptibility to *M. fructicola* artificially inoculated in peaches. An early detection of potential decay development in fruit after harvest, before storage and shelf-life, has been investigated in the past with different methods. Those most studied kept a representative fruit sample in disease conducive conditions (Zoffoli et al., 2009), or involved direct plating of plant material (Dugan et al., 2002) or induction of tissue senescence with paraquat or freezing (Holz et al., 2003). These methods, promoting the senescence of fruit or destroying the fruit's natural resistance, provoke the development of pathogens from the tissues. Although they are very popular and sometimes useful, they may be greatly affected by external factors, such as other fungal species concealing the presence of the target pathogen (Sanzani et al., 2012).

6.4 Conclusions

The results obtained in our study indicated, for the first time, the possibility after sampling of asymptomatic peach fruits in two I_{AD} classes to foresee, in the subsequent shelf-life, a higher presence of Monilinia rot in fruits belonging to the lowest I_{AD} class than to the fruits belonging to the highest $I_{AD.}$ In our work, the difference of infected fruits between class I and class II was 83% in 'Springbelle' and 97.9% in 'Redhaven' peaches. In conclusion, the I_{AD} can be regarded as an "easy to use" parameter to select peaches in view their susceptibility to brown rot during shelf-life.

6.5 References

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6.6 Figures

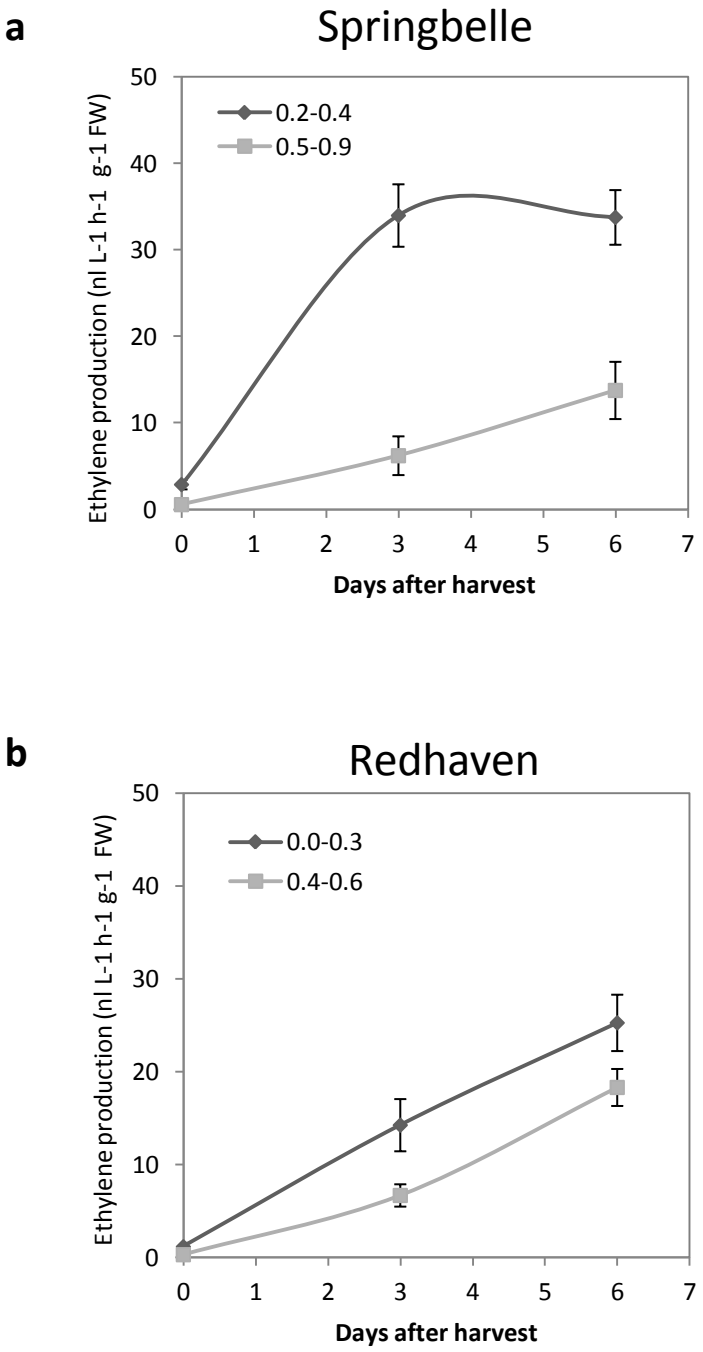

Figure 1 a, b - Ethylene production at harvest, at 3 and 6 d after harvest of peach fruit belonging to two different ripening classes for the early variety 'Springbelle' (a) and the mid-season variety 'Redhaven' (b). Each value is the mean of 5-10 fruits ± SD.

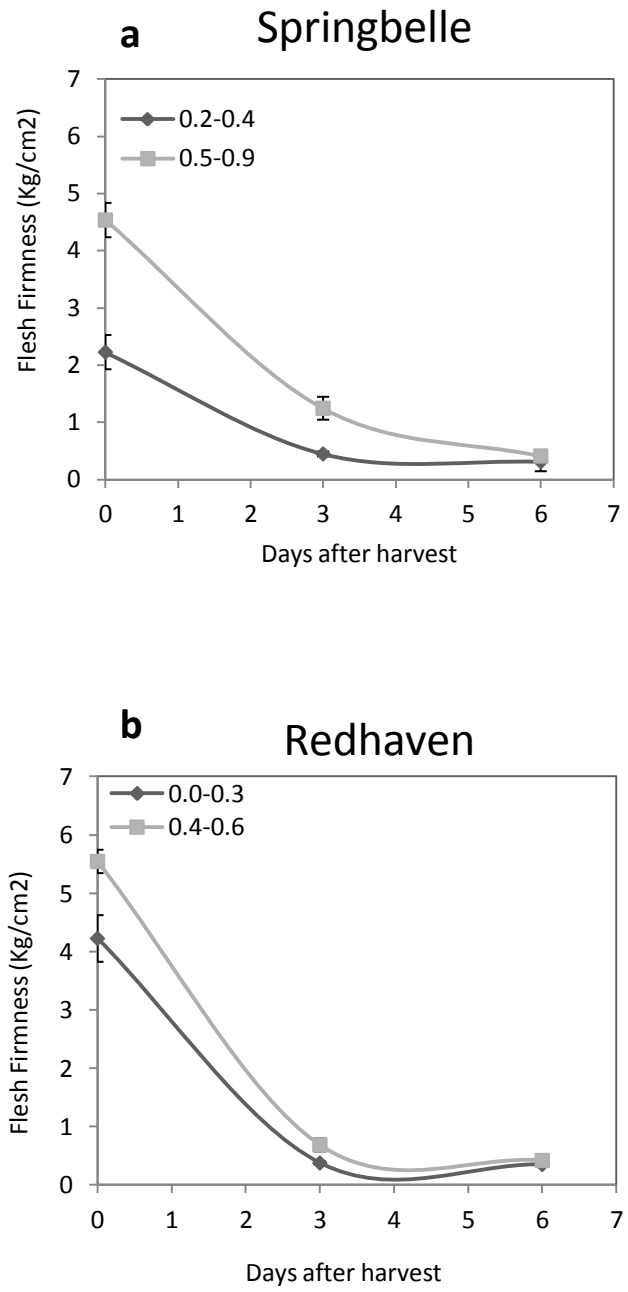

Figure 2 a, b – Flesh firmness at harvest, at 3 and 6 d after harvest of peach fruit belonging to two different ripening classes for the early variety 'Springbelle' (a) and the mid-season variety 'Redhaven' (b). Each value is the mean of 25 fruits ± SD.

6.7 Tables

Table 1

Ethylene production (nl L^{-1} h^{-1} g^{-1} FW) at harvest for each variety and I_{AD} class.

Variety	Class	I_{AD}	Ethylene production (nl L^{-1} h^{-1} g^{-1} FW)
'Springbelle'	I	0.2-0.4	2.88*±0.55a**
	II	0.5-0.9	0.56±0.14b
'Redhaven'	I	0.0-0.3	1.15±0.40a
	II	0.4-0.6	0.29±0.10b

* Each value represents the mean of 25 fruits for I_{AD} classes ± SE

**Within the same cultivar, the same letters represent no significant differences according to LSD test (P<0.05).

Table 2

Traditional quality traits: SSC (Soluble Solid Content) and TA (Titratable Acidity) for each variety and I_{AD} class at harvest (0), and after three and six days of shelf-life at room temperature.

			SSC (°Brix)			TA (g/l malic acid)		
Variety	Class	I_{AD}	0	3	6	0	3	6
'Springbelle'	I	0.2-0.4	12.7*±0.3a[z]A[y]**	12.4±0.5aA	12.1±0.4aA	9.8±0.3bA	9.3±0.5bA	7.8±0.5bB
	II	0.5-0.9	10.8±0.3bA	11.1±0.5bA	11.3±0.4bA	11.2±0.2aA	10.2±0.2aB	9.7±0.2aB
'Redhaven'	I	0.0-0.3	11.3±0.2 aA	11.1±0.2 aA	11.1±0.2 aA	7.9±0.2 aA	5.7±0.2 aB	6.0±0.1 aB
	II	0.4-0.6	10.2±0.2 bA	10.3±0.1 bA	9.9±0.3 bA	7.8±0.3 aA	5.9±0.1 aB	6.2±0.3 aB

*Each value is the mean of 25 fruits for I_{DA} classes $\pm$ SE

**Within the same cultivar, the same letters represent no significant differences according to LSD test ($P<0.05$).

[z]=Small letters represent significant differences between ripening classes within the same variety.

[y]= Capital letters represent significant differences over time within the same ripening class.

Table 3

Influence of I_{AD} on the appearance of brown rot on peach fruit. [1]Artificial infection: fruits were wounded, infected by 20 µl of conidia suspension (10^3 conidia/mL) of *Monilinia fructicola* and stored at 20°C for 3 d. [2] Natural infection: fruits were stored at 20°C for 6 d.

Variety	Class	I_{DA}	Artificial infection[1]	Natural infection[2]
			Lesion diameter(mm)	Infected fruit (%)
'Springbelle'	I	0.2-0.4	10.1*±0.4a**	60.0±3.7a
	II	0.5-0.9	7.5±0.5b	10.0±3.5b
'Redhaven'	I	0.0-0.3	13.0±1.1a	61.3±13.3a
	II	0.4-0.6	9.0±0.3b	1.3±1.3b

*Each value represents the mean of 80 fruits for I_{AD} classes±SE

**Within the same variety the same letters represent no significant differences according to LSD test ($P<0.05$)

General conclusions

In order to obtain safer fruit and vegetable, safer means of production and protection against pathogens have to be adopted. Among the new methodologies investigated, the physical methods, such as the use of heat, have acquired importance for their effects on fruit physiology. Recent results on HW treatment as alternative method for the control of postharvest fungal pathogens seem to open a new perspective in diseasesreduction. The efficacy of a hot-water treatment is determined at least by to components: 1) the direct and lethal effect of heat on pathogen that is localized on fruit surface or in the first epidermal cells and 2) the indirect effect mediated by stress-induced physiological response of the fruit (Maxin et al., 2012). The lethal effect of heating against pathogen conidia or propagules is evident but it is not clear the consequent fruit metabolism changes. In this study, *Monilinia* spp. was observed to be more susceptible to heat compared to *P. expansum*, but among the three species of *Monilinia* the greater resistance was found in *M. fructicola*. This may explain its wide diffusion in many European countries although it is still considered a quarantine pathogen.

Otherwise, the fruit species showed a different thermotolerance level. Apples were found to be more heat sensitive than peaches, in fact the temperature of 45°C was the highest tolerated without detrimental effects by apple, while peach can be treated until 60°C for 1 min without negative effects.

The use of a microarray experiment and qRT-PCR assays underlined the differences in molecular responses among peaches and apples after an HW treatment. The application of a microarray analysis on apple skin samples offered a complete information of the global gene expression of fruit treated with HW and control fruit in seven time points after the treatment within 24 hours. The results, showed an increase in expression level of *Hsps*, *Hscs*, *TFs*, *protein kinase*, *Probable pyridoxal biosynthesis protein,* etc., from 30 minutes from the treatment to 4 hours in HW treated fruit compared to the untreated. Those genes are particularly noted to be involved in acquired thermotolerance process, and we could suggest a particular role in acquired resistance also against pathogens.

The effect of heat treatment in peach fruit on some cell wall genes involved in fruit ripening such as *β-galactosidase* (*β-GAL*), *pectin lyase* (*PL*), *polygalacturonase* (*PG*) and *pectin methyl esterase* (*PME*), as well as defense related genes, *phenylalanina ammonia lyase* (*PAL*) and *chitinase* (*CHI*), heat stress-related genes as *heat shock proteins 70, 90* (*HSP70, HSP90*), and reactive oxygen species (ROS) scavenging genes was evaluated by qRT-PCR up to 6 hours from the treatment. The expression levels of cell wall genes (*β-GAL*, *PL*, *PG* and *PME*),showed a general decrease in heat treated fruit as compared to the control, whereas *PAL*, *CHI*, *HSP70* and ROS-scavenging genes increased their expression level in heat treated

samples with respect to the untreated ones. The results obtained showed a curative effect due to heat against conidia of Monilinia spp. on fruit surface as the main mode of action for the rot control in peach fruit. Contrary to what reported by several authors no resistance induction against pathogen we could suggest but slight modification at molecular level. In addition the use of SPME/GC-MS and PTR-ToF-MS detected an increase in VOCs emission on peach fruit after the heat treatment. This consequence might explain a stimulatory effect found in artificially inoculated heat treated peaches. VOCs emission from heat treated peach fruit seemed to play an important role on stimulation of conidia germination of *M. fructicola*.

From our results the HW treatment appears a promising method to control Monilinia rots of peach and nectarine, it is safe and readily available for conventional and organic production also under commercial conditions. However some aspects such as:

- time and temperature of treatment
- pathogen target
- specie

have to be considered to set up a suitable treatment protocol for a commercial applications.In addition HW treatment have a greater advantage than other non conventional control methods of fruit disease such as biological control agents and natural compounds, it does not require any registration from European Community.

In order to improve peaches quality and disease management during post-harvest phase, an innovative technique was tested. During the last years a new, portable device (Da-Meter) was introduced to monitor peach ripening from field to storage. Consequently, during the trials performed with peach fruit another important aspect emerged: the possibility to easily manage fruit ripening heterogeneity. In fact, a preliminary study on peach fruit using the I_{AD} allows to separate fruit into ripening homogeneous classes, that showed during shelf life a different susceptibility to brown rot.

As a final remarks, the strategies used in this research as the Da meter and the heat treatment are different tools with a common purposes: consumer satisfaction. The Da meter can guarantee fruit homogeneity at harvest, consequently it provides a dynamic tool for a quickly determination of the best commercial chain. The simple use of heat in particular for fruit as peaches for which no chemical treatment are allowed could prolong the shelf-life. As future prospective we will focus on the Da meter application on several varieties of peach fruit and other crops, such us cherries and table grapes, characterized by short shelf life . The first aim will be to discover, for each variety characterized by a typical I_{AD} maturity gradient, the relationship among I_{AD} and brown rot susceptibility. This could help fruit management in the

packing houses choosing which lots has to be quickly commercialized compared to others might be marketed abroad.

In addition the heat treatment will be used to reduce fungal inoculum causing grey mold for table grapes and brown rot for cherries, maintaining the quality and safety of treated fruits. Further insight at the molecular level into the interactions between host plants and new methods applied will help us to better understand the changes that occur in host plants following treatments, or the differences among fruit with different I_{AD} in the interaction with the pathogens.